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Assessment of Coliform Bacteria in Sachet Water Products Sold in Abakaliki Ebonyi State

[1]O.O. Odikamnoro, [1]C.A. Uhuo, [2]G. Gibson-Umeh, [3]B. Okeh, [4]F.N. Okoh and [5]I.M. Ikeh

[1]Department of Biological Sciences, Ebonyi State University Abakaliki, Ebonyi State Nigeria
[2]Department of Food Science and Technology, Ebonyi State University Abakaliki Ebonyi State, Nigeria
[3]Department of Applied Microbiology Ebonyi State University Abakaliki Ebonyi State, Nigeria
[4]Department of Biological Science Evangel University Ebonyi State, Nigeria
[5]Department of Zoology Nnamdi Azikiwe University Awka, Nigeria

Abstract: The study on the bacteriological quality of sachet water sold in Abakaliki Ebonyi State was conducted using bacteriological techniques. A total of 30 samples from 6 brands of sachet water products were collected from motor parks, restaurants, hawkers and market places. The samples were analyzed using multiple tube method (MTM), biochemical assays and microscopy respectively. Results were recorded as most probable number (MPN) of coliform bacteria per 100ml of water. The bacteriological quality of the samples was assessed based on the world health organization (WHO) classification system for drinking water sources. Six (20.0%) of the samples were excellent, 12 (40%) were satisfactory, 7 (23.3%) were suspicious and 5 (16.7%) were unsatisfactory using the MPN values recorded. *Escherichia coli* were identified in 5 samples (UT1 and UT2) out of the 5 were from the same brand. The level of contamination could be due to inadequate treatment of water samples by the producers, improper use of filters or post production contamination. The findings suggest the need to enforce the laws governing the operation of such production outfits and educating consumers on the need to purchase sachet water from manufactures that have been licensed to produce water and whose product bears the stamp of NAFDAC in Abakaliki.

Key words: Bacteriological · Sachet Water · Public Health · Abakaliki

INTRODUCTION

Water is a universal solvent, which consists of hydrogen and oxygen atoms. Chemically, it could be defined as a chemical substance with two atoms of hydrogen and one atom of oxygen in each of its molecules; hence the molecular formula is H_2O. Accessibility and availability of fresh clean water is a key to sustainable development and an essential element in health, food production and poverty reduction [1].

However, safe drinking water remaining inaccessible for about 1billion people in the world and the hourly toll from bacterial contamination of drinking water is 400 deaths of children below age of 5 [2]. The number of outbreaks that have been reported throughout the world demonstrated that transmission of pathogens by drinking water remains a significant cause of illness.

In Nigeria, most consumers get water supply from sources other than the Nigerian water cooperation (NWC) via their taps because only a few percentage of the total urban population is directly covered by the Nigerian water cooperation networks. The safety of the water obtained from sources other than the Nigeria water cooperation cannot be ascertained hence the water is mostly used for other household activities rather than for direct consumption. The most reliable source of drinking water is bottled water which is of better bacteriological quality [3]. But it is expensive and thus only within the means of the affluent in the society.

As an alternative, small scale industries have come up with sachet water popularly known as 'PURE WATER". This product is 60cl of water in clear nylon square sachets which have been electrically heated and sealed at both ends and widely patronized by both low

Corresponding Author: C.A. Uhuo, Department of Biological Sciences, Ebonyi State University Abakaliki, Ebonyi State Nigeria.

and middle income earners. The production of sachet water has increased tremendously with over 200 registered and unregistered producers in Nigeria.

According to National Agency for Food and Drug Administrative and Control (NAFDAC) majority of sachet water are produced under questionable hygienic environmental conditions.

Besides, some of the sachet water does not carry NAFDAC approval number. This means that they are either not registered or the producers have not completed the registration procedures of their products with NAFDAC which was established as a parastatal of the Federal Ministry of Health by Decree No. 15 of 1993 to regulate and monitor the unwholesome packaging of food and drugs administration.

Sometimes, even those who were registered have been observed to fall below the expected standard once registration have been approved [4] for example, a surveillance was carried out by NAFDAC between 2004 and 2005 revealed that some producers of packaged sachet water indulge in sharp practices such as packaging of untreated water, production under unhygienic condition, illegal production of unregistered water in unapproved premises, use of non-food grade sachets and release of these packaged water for distribution and sale without date marking [5]. Tap water is collected into a reservoir and is treated with chlorine tablet (1500 gallons to I tablet). The water is then pumped into an overhead tank through four sets of filters with pore size of 5 micron each. The water descends or flows with force into four other sets of filters, two with pore size of I micron and the other two with pore size of 0.5 micron. The water then passes through carbon into a stainless steel ultra violet machine before finally passing through a packaging machine where it is automatically packed into sachets (60cl). In built in the machine is an ultra violet light that cast on the role of the rubber for packaging.Sachet or packaged water is any water that is produced, distributed and offered on scale either scaled in food grade or other containers intended for human consumption [6, 7].

The request for portable drinking water has led both income entrepreneurs and large scale investors to venture into the production of portable water. Such portable water include Gospel, Aqua Rapha, A-zed, Tek pack, Utomic and E&E etc and more than one thousand brands of sachet water all over the country.

This study was aimed to determine the bacteriological and parasitological quality of sachet water popularly known as “Pure water” produced and sold in Abakaliki and its public health importance.

Study Area: Abakaliki, capital of Ebonyi state is located in the Southeastern Nigeria. It lies at the intersection of roads from Enugu, Afikpo and Ogoja. It is located at latitude 6.3333°N and longitude 8.1169°E. This town was selected for this investigation because it is well noted for its water lack and or shortage. The water transported in tankers is of questionable quality hence is mostly used for house hold activities while sachet water is believed to be pipe-borne water that has undergone further treatment is relied upon by many residents for the purpose of drinking. With the obvious demand for pure water, small scale industries have sprang up overnight producing sachet water to meet the demand. The different brands of sachet water (Aqua Rapha, Tek Pack, E & E, Utomic, A-Zed and Gospel) were collected and taken through bacteriological examination. Analysis was to determine Most Probable Number (MPN) of coliform organisms per milliliter of sachet water as well as to determine the presence of pathogenic bacteria as *Escherichia coli*.

Sample Collection: The study was carried out between December 2013 and January 2014. A total of 30 samples of 6 brands of NAFDAC registered sachets were collected from Abakaliki area and analyzed. 5 replicate samples of each brand were collected. The collection was made randomly from hawkers, provision stores, market places, restaurants and motor parks. This was to ensure that the analysis covers a wide range in the area statistically. The samples analyzed are shown below (Table 1) with their place of production and NAFDAC registration number.

Inoculation and Incubation: One end of each sample of sachet water was cleaned with 70% ethanol. A sterilized pair of scissors was used to cut open each sachet of water at the sterilized end. Ten milliliters (10ml) of sample was measured with a sterile 10ml disposable pipette and aseptically dispensed into each of the five tubes containing 10ml of double strength purple Mac-Conkey broth each with an inverted Durham tube. The tubes were closed tightly and then shaken to distribute the sample uniformly throughout the medium and then incubated at 37°C. The chamber was always disinfected with 70% alcohol before and after the analysis.

After 24-48 hours, the tubes from the presumptive fermentation test showing gas and acid formation were recorded and corresponding Most Probable Number (MPN) index was determined from the probability table (Mc-Crady). A sterile pipette was used to transfer 1ml of the culture from the positive

Table 1: NAFDAC registered sachet water samples

Brand Name	Code	Volume	NAFDAC number	State/town of production	Batch no	Production date	Exp. date
Aqua Rapha	AR	60cl	01-4087L	Enugu	Nil	Nil	
Tek Pak	TP	60cl	A1-0189L	Enugu	Nil	Nil	
Gospel	GP	60cl	01-1986L	Abakaliki	Nil	Nil	
Utomic	UT	60cl	A1-0556L	Afikpo	Nil	Nil	
E & E	EE	60cl	B1-0657L	Ohaozara	Nil	Nil	
A-zed	AZ	60cl	01-1993L	Afikpo	Nil	Nil	

presumptive fermentation tubes into tubes containing 5ml brilliant green lactose bile broth aseptically and incubated for 24-48hrs at 37°C.

Following incubation, aliquots from the cultured positive tubes were aseptically streaked on Mac-Conkey agar for total coliform and Eosin Methylene Blue agar (EMB) for faecal coliform and incubated at 37°C and 44°C, respectively. Suspected colonies of coliform and faecal coliform were identified through biochemical tests utilizing Triple Sugar Iron (TSI) agar, IMViC tests including Indole,MR, Voges Proskaüer and Simons Citrate utilization,Motility (Peptone water) and Urea agar.

Gram Staining: Immersion oil was applied on the Gram stained slide and viewed microscopically using X100 objectives lens under microscope.

Motility Test: It was examined under a microscope using x10 and x 40 objective, movement of visible organism were identified as being motile [8].

Inoculation on Slope Cultures (TSI Agar, Simmons Citrate Agar and Urea Agar): We inoculated slope and butt by using a sterile straight wire to stab into the butt first, then used the same wire to streak the slant in a zigzag manner [9].

Statistical Analysis: All the data collected were analyzed by calculating the percentages which are presented on tables. The Chi-Square was also calculated to determine the bacterial contaminants of the analyzed sachet water.

RESULTS

The water samples analyzed in this study were exclusively for bacteriological contamination of Indicator organisms e.g. *E. coli.*

The bacteriological analysis of 6 common brands of sachet water sold in Abakaliki showed that most of the sachet water sold in Abakalikiproduced bacteria growth. In this examination, the most probable organisms obtained were *Escherichia coli, Shigellaspecies, Klebsiellaspecies* and *Enterobacter species*.

Table 2: Most Probable Number (MPN)of positive samples on Mac-Conkey Broth.

S/N	Sachet water brand	Number of tubes showing fermentation/gas production	MPN/100ml
1	TP_1	1	1
2	TP_2	2	2
3	TP_3	4	5
4	GP_1	4	5
5	GP_2	3	4
6	GP_3	2	2
7	AR_1	1	1
8	AZ_1	1	1
9	AZ_2	1	1
10	EE_1	1	1
11	EE_2	2	2
12	UT_1	3	4
13	UT_2	2	2
14	UT_3	4	5
15	UT_4	2	2
16	AR_2	2	2
17	EE_3	2	2
	Total	37	42

Table 3: Colour and colonial characteristics of the isolated bacteria on Eosin methylene blue agar and Mac-Conkey agar

Colony characteristics/colour		
Eosin methylene blue agar (EMB)	Mac-Conkey agar	Most probable organism
Large blue-black, green metallic sheen.	Pink	*Escherichia coli*
Large, mucoid, blue-black	Mucoid pink	*Enterobacter/ Klebsiella*
Large Colourlessto amber	Colourless	*Shigella*

Table 4: Results of biochemical tests of different samples of sachet water

				TSI				
S/N	Water sample	Motility	Gram +ve reaction	Slant	Butt	Urea	Citrate	1ST Organism isolated
1.	GP_1	-	-	R	Y	-	+	*Shigella*
2.	GP_2	+	-	Y	YG	-	-	*E. coli*
3.	GP_3	-	-	Y	R or YG	+	+	*Klebsiella*
4.	AR_1	+	-	Y	R	-	-	*Enterobacter*
5.	TP_1	+	-	Y	YG	-	-	*E.coli*
6.	TP_2	+	-	R	Y	-	+	*Shigella*
7.	TP_3	-	-	Y	R	+	+	*Klebsiella*
8.	AZ_1	-	-	R	Y	-	+	*Shigella*
9.	AZ_2	+	-	Y	R	-	-	*Enterobacter*
10.	EE_1	+	-	Y	YG	-	-	*E. coli*
11.	EE_2	+	-	R	Y	-	+	*Shigella*
12.	UT_1	+	-	Y	YG	-	-	*E. coli*
13.	UT_2	-	-	R	Y	-	+	*Shigella*
14.	UT_3	+	-	Y	YG	-	-	*E. coli*
15.	UT_4	+	-	R	Y	-	+	*Shigella*
16.	AR_2	-	-	R	Y	-	+	*Shigella*
17.	EE_3	+	-	Y	R	-	-	*Enterobacter*

Key:
R = red
Y = yellow
YG = gas formed
- = negative
+ = positive

Table 5: Classification of the samples according to (WHO, 2007) criteria for drinking water

Class	Grade	Presumptive count per (100ml)	Number of sample n= 30	Percentage (%)
I	Excellent	0	6	20%
II	Satisfactory	1-3	12	40%
III	Suspicious	4-10	7	23.3%
IV	Unsatisfactory	>10	5	16.7%

Table 6: Result of Chi-square analysis of the bacteria contaminants of sachet water in Abakaliki metropolis.

Water brand	Contaminated sample	Uncontaminated sample	Degree of freedom	% error	X^2(cal)	X^2(tab)
AR	2	3	5	0.05	2.291	11.1
TP	3	2	5	0.05	2.291	11.1
GP	3	2	5	0.05	2.291	11.1
UT	4	1	5	0.05	2.291	11.1
EE	3	2	5	0.05	2.291	11.1
AZ	2	3	5	0.05	2.291	11.1

H_O: there is bacterial contamination in sachet water in AI metropolis
Hi: there is no bacterial contaminant in sachet water sold in AI metropolis.
Decision rule:
X^2cal< X^2 tab = H_O is accepted
X^2cal> X^2 tab = H_O is rejected
Decision
Since X2 (cal) is less than X2 (tab) HO is accepted; there is bacteria contaminants of sachet water in Abakaliki metropolis

DISCUSSION

Access to good quality drinking water is a challenge in most towns and cities in Nigeria and households have for years depended on other sources of water to supplement their activities. The introduction of sachet water to consumers was to provide safe, hygienic and affordable drinking water to the public. Although this is a laudable idea, current trends seem to suggest that sachet drinking water could be a route of transmission of diseases. This study evaluated the Bacteriological Contaminants of Sachet water in Abakaliki Metropolis.

The 30 samples based on the MPN values were classified as excellent, satisfactory, suspicious and unsatisfactory. The WHO guidelines for drinking water states that for treated water no more than 1 of 10 analytical units should have an MPN value of >2.2MPN/100ml of water.

The total coliform count of the various brands was found to be high and this agrees with a similar study done in South-Eastern Nigeria (Aba and Owerri) [10] in (2003). The level of coliform bacteria in the sachet water from the various brands sampled did not meet the WHO guidelines for drinking water [11]. When water supplies contain coliform bacteria in levels greater than one per 100ml of water, the water may also contain pathogens that cause acute intestinal illness. While generally considered a discomfort to health, these infections may be fatal for infants, the elderly and those who are sick [12].

The coliform positive samples were also tested for faecal coliform, which also tested positive. Faecal coliform is considered more as an indicator of faecal contamination because whereas coliform can exist in the environment, faecal coliform are non-disease causing organisms which are found in the intestinal tract of warm blooded animals hence its presence is indicative of pollution with animal or human waste Standard Methods for the Examination of Water and Waste water, (2005). They are primarily used to indicate the presence of bacterial pathogens such as *Salmonella spp, Shigellaspp, Vibrio cholerae, Campylobacter jejuni, Campylobacter coli, Yersinia enterocolitica* and pathogenic *E. coli.* These organisms can be transmitted via the faecal/oral route by contaminated or poorly treated water and may cause diseases, such as gastroenteritis, salmonellosis, dysentery, cholera and typhoid fever.

The presence of faecal coliform observed in packaged water has been reported to be due to poor hygienic practices of producers, failure to wash hands, ignorance about good hygienic practices as well as the presence of animals in the vicinity of the factory [13].

All the faecal coliform positive samples were tested for *E. coli* and five of them were found to be positive. The presence of *E. coli,* a faecal coliform is a strong indicator of recent sewage or animals waste contamination. Treated water should not contain this organism because it is also an indicator for other pathogens that may be present in faeces (US Environmental Protection Agency, 2003). *E. coli* and coliforms above the WHO standards were also reported from a Port Harcourt, Nigeria study that fielded five brands of sachet water [14].

Water that has been treated for the purpose of drinking should not be contaminated with coliform, if present then the source of the contamination must be located. Sachet water is sold to the public for direct consumption hence it is supposed to undergo treatment. In Nigeria, the water source for the production of the sachet water is pipe borne water supplies which is then supposedly further treated to make it safe for direct consumption hence sachet water is regarded as treated water. The source of water for sachet water, therefore, cannot be compared to natural mineral waters, which are the usual source for bottled water in the developed world, which may not be entirely free of bacterial contamination [11]. Most bacteria are thought to enter as contaminants during bottling or bagging.

In the case of sachet water, the sources of contamination could be the main water source because it is reported that some unscrupulous producers just bag and seal pipe water without any form of treatment and further processes of purification [6,8]. This could also be found in Abakaliki as most of the so called sachet water emerges from the premise which does not have any proof of water production setting thereby posing public health danger to the consumers.

Poorly maintained filter systems are also a possible source of contamination because bacteria can grow on filters if these are not changed regularly and thereafter enter the water supply. It has been shown that charcoal filters used in removing unpleasant odours from drinking water can support large bacterial numbers. Bacteria count as high as 7 X 10^6/ml were detected in the effluent from a charcoal filter 6 days after installation [5]

My findings show that it is necessary to determine the presence of faecal coliform from water intended for drinking in order to prevent faecal coliform on regular basis. It could be deduced that Aqua Rapha and A-zed are of good quality for human consumption despite finding a few coliforms in one of the batches, this shows some degree of high standard of sanitary procedure in production as other sachets were devoid of fecal coliforms.

This should not be ignored to avoid subsequent contamination of some other batches in the near future. Sachet water from Utomic, E & E, Tek pack and Gospel were of relatively poor sanitary standard as much of the batches were contaminated with faecal coliform, this shows that they are not fit for human consumption. It was generally observed that loaders employed to package the water were seen unhygienic, sweating into the water and various stalls were located outside the houses without cover. Therefore, these practices could predispose the water to contamination leading t infection in drinking.

CONCLUSION

The primary objective of monitoring drinking water is to protect the health of the community by preventing the spread of water borne diseases. The peculiar situation of sachet water is that most of the producers are not registered hence monitoring becomes difficult. The NAFDAC of Nigeria as the regulatory authority should therefore insist on official registration of all producers and publish regularly the list of producers who have registered their products, conduct routine tests on these products and alert consumers about those with the unwholesome products. Most households in Ebonyi now rely on sachet water as their main source of drinking water hence if contaminated products get onto the market, the consequences could be fatal.

It is obvious from the study that some of the sachet water being sold in Abakaliki and environs may be safe as far as bacteriological quality is concerned but a lot of more of these products are unsafe for drinking. Regrettably the consumer has no way of knowing which product is safe and which one is not. It therefore behooves the regulatory authorities to employ adequate measures to protect the consumer because sachet water has come to stay and the producers are increasing by the day.

Recommendation: It is recommended that producers of packaged water should endeavor to disinfect their products with solar radiation which is simple to construct and easy to maintain. Better purification of water prior to packaging should be emphasized, formal workshops should be held at local sites of production to educate the staff on the need for good hygienic habits and proper handling of working tools.

NAFDAC officials should also carry out unannounced inspection on sites of production of already registered sachet water. Unless the situation is rectified on the count with particular reference to adequate adoption of sanitary measures for provision of portable water, the problem would not be over the future as wished.

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