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BACTERIOLOGICAL STUDIES ON SATCHET WATER SOLD IN ABA, ABIA STATE NIGERIA

Chima O. C.[1*], Opara A. U.[1], Dike-Ndudim J. N.[1], Nwosu D. C.[1], Ahiara C. O.[1], Ibekwe C. N.[1], Nwanjo U. B. C.[2] and Nwogwugwu N. G.[3]

[1]Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria.
[2]Imo State Government Specialist Hospital Umuguma, Imo State, Nigeria.
[3]Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Rivers State, Nigeria.

***Corresponding Author: Chima O. C.**

Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria.

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ABSTRACT

Bacteriological studies on sachet water samples sold in Aba metropolis, Abia State, was undertaken to determine their portability for human consumption. Sachet water samples from twenty five (25) of the fifty (50) registered sachet water factories were collected at random from vendors at market places, Schools, Campuses, Motor parks, Recreation parks and Streets in Aba metropolis. The samples (five from each brand) were used and analyzed within 6 hours of collection. Four weeks later, a second batch of such samples from the same factories (also five from each brand) were collected and treated in like manner using pour plate method with molten MacConkay agar. After solidification, the plates were incubated at 37^0C and 22^0C for 24 – 72 hours, after which colony counts were conducted on the culture plates that had growths. The mean plate count was taken and the organisms from each water sample were identified based on morphological and biochemical characteristics. The result shows that three sachet water brands (12%), had growths of pathogenic organisms after 24hrs of incubation at 22^0C and 37^0C in the first batch, while, five sachet water brands (20%) had growth in the second batch. The organisms isolated were *Klebsiella pneumonia, proteus mirabilis*, and *pseudomonas eruginosa*. Hence, some of the sachet water packs sold in Aba metropolis were found to be unfit for human consumption. The results from this study shows that further purification is necessary before consumption and that the attention of NAFDAC and other authorized bodies be drawn to those factories whose water samples are contaminated for necessary action.

KEYWORDS: *bacteriological studies, satchet water, Aba.*

INTRODUCTION

Rivers, streams, lakes and other sources of water receive domestic and industrial wastes from various sources. With the increasing rate of water pollution, the slogan by most scientists in water bacteriology that "the solution to pollution is dilution" cannot be applied any longer (Ogbulie *et al.*, 1998).

Today, in developing countries, especially in Nigeria, the need for safe drinking water is highly desirable, the reason being that the incidence of water borne diseases in our society today is alarming and could be fatal (Opara *et al*., 2011).

For many years now sachet water, as a drinking water has become a booming business in Nigerian cities. This may be attributed probably to its affordability when compared to bottled water. Moreover, water co-operation in some cities in Nigeria have failed in municipal water supply.

As a result, sachet water packagers and owner managers have continued to exploit the opportunity to give the public the sachet water or the so-called "Pure water".

Unfortunately, many of this sachet water may be potential sources of water borne diseases to consumers. Typical examples of the diseases that can be acquired by drinking impure sachet water include typhoid fever, cholera and gastroenteritis. These water borne diseases are evidently not uncommon in our society today and they threaten human life (Nwaedozie, 2000).

Therefore, to minimize and possibly to eliminate the occurrence of water borne diseases, transmissible through the use of sachet water, the need to ascertain the purity and fitness of the so-called "Pure water" is of great importance.

The guideline for the production of portable drinking water as stipulated by (WHO, 1996), should be applied and is as follows. It stipulates the use of the under listed equipments and materials for portable water production

namely; Water from a bore-hole, Water tank, composite filter, Cartridge filter, others include, Ultra-violet water sterilizer, Automated sealing and Cellophane bag.

Water from a borehole or any other source is stored in the water tank from which it is being channeled to the composite filters. The composite filters are divided into two separate filters. The first containing layers of white sand that filters out sand from the water. After the filtration of sand, the water goes into the other filter that contains activated carbon. The carbon filter removes taste, colour and odour from the water. The water then goes into the cartridge filters through the connecting tube. The cartridge filters consist of five separate filters distinguished by their sizes in micrometers; 5, 2, 1, 0.5. The remaining one of these filters carries out further the work done by the carbon filter, the other four, filter out invisible dirt that may be present in the water.

From the cartridge filters, the water goes to the ultra–violet water sterilizer that kills microorganisms using ultra–violet radiation. After sterilization by the ultra–violet sterilizer, it is expected that a quality control be done to determine the purity of the processed water. The water is than channeled to the automatic sealing machine that sterilizes the cellophane bag, packages and seals the water. The pH level of the purified water should range from 6.5 to 8.5 otherwise; it should be adjusted by adding lime or using any other appropriate method (WHO, 2004).

Since microorganisms are ubiquitous in nature, sachet water is not an exception to contamination. The aim of this research therefore is to ascertain the portability of the sachet water offered to the public in Aba metropolis Abia state.

MATERIALS AND METHOD

STUDY AREA

The Study was undertaken in Aba metropolis, Abia State Nigeria between October and December 2015.

Aba is a city in the southeast of Nigeria and the main trading centre in Abia State. Aba metropolis is made up of two local government areas namely Aba south and Aba North. It lies along the west bank of the Aba River, and is at the intersection of roads leading to Port Harcourt, Owerri, Umuahia, Ikot Ekpene, and Ikot Abasi. Aba is a major urban settlement and commercial centre in a region that is surrounded by small villages. Aba is well known for its craftsmen. As of 2014 Aba had an estimated population of 2,200,000.

SAMPLE COLLECTION AND SAMPLE SIZE

There are about 50 registered sachet water factories in Aba, Abia State and 50% of this population was used in the investigation. Hence, 25 brands of sachet water samples (5 from each brand), was collected at random from market places, schools, campuses, motor packs, on the Roads and Streets in Aba metropolis.

The brand names of the sachet table water collected are listed below.

Trixi table water, Uloma, Vic Nic, Elis, Avec, Evita, OBK , Stan, others are Nicho D, V C, Nneoma, Lizzy, Gihwat, Predap, DINS, Mei Eden, T-Maje, also included are Beeds, O.G, Above All, Tina, Jeobic, Neu-Life, Dechus and Lawsco table water.

The samples (five from each brand) were analyzed within 6 hours after collection.

Two weeks later a second batch of the samples (also five from each brand) were collected. The medium used for the investigation is MacConkey medium. It was prepared according to the manufacturer's instructions.

TEST PROCEDURE

The method used is the surface plate count (viable count) as described by Ochei and Kolhatkar, (2007), Topley and Wilson, (1989).

1ml from each water sample was dispensed aseptically into petri dish containing 15mls of molten MacConkey agar in duplicate and properly mixed by rotating three times clockwise, anticlockwise, forward and backwards direction and allowed to solidify.

After solidification, the plates were incubated at 37^0C and 22^0C for 24-72 hours. After 24hr, viable plate count was done on those plates that yielded growths, those without growth were incubated further. At the end of incubation, colony count was done on each culture plate and the mean plate count was taken. The organisms isolated from each water sample were identified morphologically and biochemically.

STATISTICAL ANALYSIS USING CHI SQUARE METHOD

Batch 1

Brands	Growth at 37^OC	Growth at 22^OC	Mean plate at 37^OC	Mean plate at 22^OC
Uloma	5	5	4	3
O.G	3	4	5	4
Tina	5	5	6	4

Batch 2

Brands	Growth at 37^OC	Growth at 22^OC	Mean plate at 37^OC	Mean plate at 22^OC
Uloma	5	5	4	6
Nicho D	2	3	3	3
Mei Eden	1	2	2	3

O.G	5	4	3	6
Tina	5	5	5	4

(OBSERVED FREQUENCY O_f)

Batch	Growth at 37^OC	Growth at 22^OC	Mean plate at 37^OC	Mean plate at 22^OC	Row total
1	13	14	14	11	52
2	18	19	17	20	74
Column Total	**31**	**33**	**31**	**31**	**126**

$$X^2 = \sum_{O=1}^{m}\sum_{E=1}^{n}\frac{(O_f - E_f)^2}{E_f}$$

where O_f = Observed frequency, E_f = Expected frequency

$$= \sum_{O=1}^{2}\sum_{E=1}^{4}\frac{(O_f - E_f)^2}{E_f}$$

m = number of rows, n =number of columns

$$E_f = \frac{\text{Row total} + \text{Column total}}{\text{Grant total}} = E_{ij}$$

Where i = row position (1^{st}, 2^{nd})

j = column position (1^{st}, 2^{nd}, 3^{rd}, 4^{th})

E1.1 = $\frac{52 \times 31}{126} = 12.79$ E1.2 = $\frac{52 \times 33}{126} = 11.79$

E1.3 = $\frac{52 \times 31}{126} = 12.79$ E1.4 = $\frac{52 \times 31}{126} = 12.79$

E2.1 = $\frac{74 \times 31}{126} = 18.21$ E2.2 = $\frac{74 \times 33}{126} = 19.39$

E2.3 = $\frac{74 \times 31}{126} = 18.21$ E2.4 = $\frac{74 \times 31}{126} = 18.21$

O_f	E_f	$O_f - E_f$	$(O_f - E_f)^2$	$(O_f - E_f)^2/E_f$
13	12.79	0.21	0.04	0.003
14	11.29	2.71	7.34	0.650
14	12.79	1.21	1.46	0.114
11	12.79	-1.79	3.20	0.250
18	18.21	-0.21	0.04	0.002
19	19.39	-0.39	0.15	0.008
17	18.21	-1.21	1.46	0.080
20	18.21	1.79	3.20	0.176
TOTAL				**1.283**

$$X^2 = \sum \frac{(O_f - E_f)^2}{E_f} = 1.283$$

X^2 cal. = 1.283

DEGREE OF FREEDOM, DF

Degree of freedom, df = $(r - 1)(c - 1)$

Where : r = number of rows

c = number of columns

X^2 tab. (0.05) = 7.815

Chi square tabulated with 3 degrees of freedom at 0.05 level of significance = 7.815.

Decision: Since X^2cal < X^2 tab, we accept the null hypothesis that there is prevalence of Coliform bacteria of health importance in some of the sachet water being sold in Aba metropolis.

RESULTS

From the examination of the first batch of 25 samples, 22 brands (88%) yielded no growths after 72 hours of incubation at 37^0C and 22^0C. The remaining 3 brands (12%) had growths after 24hours of incubation at 22^0C and 37^0C. The mean plate count and organisms isolated are shown in tables 1 and 2 respectively. Uloma and Tina table water brands yielded 100% bacteria growth at 37℃ and 22℃ while O. G. table water was not consistent at both temperatures.

The result of the second batch investigation carried out after 4 weeks are shown in tables 4 and 5. It shows that, Uloma, Tina and O.G. table water samples were similar to those of the first batch investigation. In the second batch, 20 brands (80%) yielded no growth after 72 hours of incubation. The remaining 5 brands, (20%) yielded growth. It is important to note that the organisms that were isolated grew at both 22^0C and 37^0C because they are members of Enterobacteriaece and were capable of growth at both temperatures. From both batches the organisms isolated were *Klebsiella Pneumoniae, Pseudomonas aeruginosa, and Proteus mirabilis*. In the first batch, *Klebsiella pneumoniae* predominated while *Proteus mirabilis* predominated in the second batch.(Tables: 4.3 and 4.6).

Table 1: Number of samples with growth in batch one.

Brands with growth	No of samples Examined	No with growth at 37℃	No with growth at 22℃	Mean plate count at 37℃	Mean plate count at 22℃
Uloma	5	5	5	4	3
O.G	5	3	4	5	4
Tina	5	5	5	6	4

Table 2: Bacteria Isolated in batch one.

Brands with growth	Bacteria isolated at 37℃	Bacteria isolated at 22℃
Uloma	*Klebsiella pneumonia*	*Klebsiella pneumonia*
O.G	*Klebsiella pneumonia, Proteus mirabilis*	*Klebsiella pneumonia*
Tina	*Pseudomonas eruginosa*	*Pseudomonas eruginosa, Proteus mirabilis*

Table 3: Relative abundance of isolated bacteria in batch.

Bacteria isolated	NO at 37℃ and 22℃	Percentage
Klebsiella pneumonia	4	8%
Proteus mirabilis	2	4%
Pseudomonas eruginosa	2	4%

Table 4: Number of samples with growth in batch two.

Brands with growth	No of Samples Examined	No with growth at 37℃	No with growth at 22℃	Mean plate count at 37℃	Mean plate count at 22℃
Uloma	5	5	5	4	6
Nicho D	5	2	3	3	3
Mei Eden	5	1	2	2	3
O.G	5	5	4	3	4
Tina	5	5	5	5	4

Table 5: Bacteria isolated in batch two.

Brands with growth	Bacteria isolated at 37℃	Bacteria isolated at 22℃
Uloma	*Klebsiella pneumonia*	*Klebiella pneumonia*
Nicho D	*Proteus mirabilis*	*Proteus mirabilis*
Mei Eden	*Proteus mirabilis*	*Proteus mirabilis*
O. G.	*Klebsiella pneumonia, Proteus mirabilis*	*Klebsiella pneumonia*
Tina	*Pseudomonas eruginosa*	*Pseudomonas eruginosa, Proteus mirabilis*

Table 6: Relative abundance of isolated bacteria in batch two.

Bacteria isolated	No at 37℃ and 22℃	Percentage
Proteus mirabilis	6	12%
Klebsiella pneumonia	4	8%
Pseudomonas eruginosa	2	4%

Table 4.7: Characteristic And Identity Of Isolates.

Isolates	G.R	Rod	Cocci	Mot	Cat	Oxid	Indo	M.R	V.P	Cit	Ur	Sp	Glu	Lac	Suc	Indentity of Isolates
1	-	+	-	-	-	-	-	-	+	+	+	-	A/G	A	A	*Klebsiella pneumonia*
2	-	+	-	+	+	-	-	-	-	-	+	-	A/G	-	-	*Proteus mirabilis*
3	-	+	-	+	+	+	-	-	+	+	+	+	-	A	-	*Pseudomonas eruginosa*

Keys: + = Positive, - = Negative, A/G=Acid/Gas, A=Acid, G.R = Gram Reaction, Rod=Rod shape, Cocci=Cocci shape, Mot=Motility, Cat= Catalase, Oxid=Oxidase, Indo= Indole, M.R=Methyl Red, V.P=Voges proskauer, Cit=Citrate, Ur=Urease, Sp=Spore, Glu=Glucose, Lac=Lactose, Suc= Sucrose.

DISCUSSION

Bacteriologically, the quality of some of the sachet water sold in Aba, Abia State, where found to be very poor because of certain pathogenic organisms that were isolated from them. Out of 25 brands examined, only 5(20%) from the first and the second batch, had growth with 3 brands (12%) been 100% consistent in contamination which fall short of the WHO standard for drinking water (WHO, 1984). All the organisms isolated were members of the family *Enterobacteriaceae* and are highly noted as being responsible in water borne diseases (Opera *et al.*, 2011). According to Uwaezuoke *et al.* (2003) and Dike *et al*. (2015) they are indigenous to natural water, soil, vegetations (plants) and in the intestinal tracts of humans and animals. Those non indigenous to natural water, according to Adesiyum (1993) and Dike *et al*. (2007) are washed into water source by urban and rural run-off from their natural habitats in the soil, and on vegetations, from human and animal wastes deposited randomly on land or in concentrated confinement. The organisms isolated were really baffling for according to Topley *et al*. (1989), these organisms should not be present when water is properly treated.

The presence of the identified organisms in the treated water (sachet water) may be attributed to one or more of the following.

- Contamination of the treated water by organisms' harbored in the connecting tubes to the packaging machines.
- Lack of or poor quality control system, otherwise the level of contamination of the source of water could have been identified before packaging.
- Poor or faulty treatment mechanism, it is possible that the equipment or machine used in the purification process is not functioning effectively.
- It is also possible that the hawkers of those contaminated sachet water could do manual packaging of untreated water at home using the company's pre-produced water bags.

The possible health hazard of drinking contaminated or poorly treated water is tremendous. According to Ogedengbe (1986) and Dike *et al*., (2015), infectious diseases such as gastroenteritis, diarrhea, typhoid fever, dysentery, cholera, and some other intestinal tracts disease could be acquired or transmitted to man through drinking water.

Also, Skirrow, (1997), reported the outbreak of epidemic diseases as a result of drinking water. It is also important to mention here that the finding suggests that some of the sachets water sold in Aba do not conform to the international standard. This follows from the view of Dike, *et al*., (2015) ,that, for any water to conform with the international standard such water, must be safe biologically, chemic ally, and aesthetically. Biologically, therefore, some of the sachet water is not portable since pathogenic organisms were isolated.

CONCLUSION

If all the processes of sachet water production stated in this work were followed strictly, there would have been complete elimination of the organisms from the water during the process of production. Since this is not the case, the consumer protection agencies therefore should not only stop at issuing registration numbers to sachet water producers, they should also ensure an effective week to week quality control measures of these sachet water been sold.

Also, they should frequently inspect the source of water used by the sachet water producers, the efficiency of their production processes and the installation of the water supply system with the objective of identifying potential risk of contamination and recommend remedial measures.

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