

MICROBIOLOGICAL EXAMINATION OF SACHET WATER SOLD IN ABA, ABIA – STATE, NIGERIA

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This study was carried out to determine the microbial quality of 10 randomly selected brands of sachet water sold at major Markets and Motor parks in Aba town, south east of Nigeria. Twenty water samples collected and analysed using standard microbiological tests. Heterotrophic bacterial count, presumptive (total coliform and faecal coliform counts) tests, confirmatory tests (total coliform and faecal coliform counts) and culture were performed to determine the identities of the isolates. Water sample F had the highest total heterotrophic bacterial count (THBC) of 4.7×10^6 cfu/ml while water sample J with THBC of 0.2×10^6 cfu/ml had the lowest contamination level. Water samples A, B, C, E, H and I had the highest Total Coliform Bacteria (MPN/100ml) of 5 while sample D with 0 was the least. A large proportion (32%) of the water samples analysed tested positive for *Staphylococcus* spp. while *Enterobacter* spp. was the least isolated pathogen. The result of this study indicated that 5 of the 10 brands of sachet water sampled in Aba town met the WHO standard for drinking water quality. These results raised a question of safety and it is therefore suggested that regular analysis of sachet water would greatly improve public health.

Keywords: Micrbial quality, Satchet water, Heterotrophic bacterial, coliform.

INTRODUCTION

Water is indispensable for life. However, it is estimated that about 1.2 billion individuals worldwide do not have access to portable water (Third World water Forum on water, 2003). In many developing countries, availability of water has become a critical and urgent problem and it is a matter of great concern to families and communities that depend on non-public water supply system (Okonko *et al.*, 2008). Increase in the human population has exerted an enormous pressure on the provision of safe drinking water in developing countries (Umeh *et al.*, 2005). To curb this health problem, bottled water was introduced, but only individuals who have a good financial status can afford these products. Low income earners are left with no option but to consume sachet packaged water that is cheaper. It is readily available and affordable; sachet water is sold in most road side vending food stalls

in Nigeria. Small nylon sachets which are electrically heated and sealed at both ends are used to package water 0.5 litres of water and these were introduced into the market in Nigeria. There are many different brands of sachet drinking water that are beautifully packaged, properly labeled and advertised (Ekwunife, *et al.*, 2010). Although these products are popularly termed "Pure Water", they are usually not free of microbial contaminants (Caroli *et al.*, 1985; Omemu *et al.*, 2005; Taura *et al.*, 2005; Ezeugwunne *et al.*, 2009; Oladipo *et al.*, 2009). Occasionally, contamination of sachet water may occur either during the processing, transportation or improper handling by hawkers. Moreover, a greater proportion of the water that is used for the production of sachet water is obtained from boreholes that is exposed to microbial contamination through rainfall runoffs and the

fact that they are usually constructed very close to pit toilets.

The aim of this study is to isolate and identify bacteria from sachet water sold in Aba and to determine the health and socio economic implication of sachet water sold in Aba .

MATERIALS AND METHODS

Sampling site

Sachet water samples were bought from road side vendors in Aba, Aba State, Nigeria. Aba is a commercial town in Aba-State, South-East Nigeria. The town lies on the longitude 30 East of Greenwich Meridian and latitude 25 north of the Equator. Aba has international recognition due to the presence of small scale industries and a number of local markets. In Aba there is lack of pipe-borne water, improper water management and purification systems. Moreover, there is lack of proper environmental sanitation. Against this backdrop the commercialization of sachet water was implemented with the intention to supply portable and treated water to its inhabitants.

Collection of Samples

A total of 20 sachets water from 10 most popular brands that were randomly selected were bought from water vendors at major markets and motor parks in Aba town. Samples were properly labeled and transported to the Laboratory on ice for bacteriological analysis. Upon arrival in the laboratory samples were analysed within two hours. The water in each sachet was mixed by moving the up and down. Cotton wool was soaked in 70% (v/v) ethanol and used to wipe a small portion of the sachet before opening with a pair of sterile sharp scissors.

Samples analysis

To analyze samples ten fold serial dilutions were prepared and 100µl aliquots from dilution was spread plated on standard bacteriological culture media for enumeration of the different microbial counts. The plates were prepared in duplicates to ensure accuracy.

Total Heterotrophic Bacterial Count

Nutrient agar (Biotec, United Kingdom) plates were used to determine the total heterotrophic bacterial counts and the plates were incubated for 24h at 37°C. After incubation, the number of colonies on each plate was counted and averages were computer for each sample. The number of bacterial contaminants were determined as previously reported (Cheesbrough, 2000).

TOTAL COLIFORM AND FAECAL COLIFORM COUNT

PRESUMPTIVE TEST

The Most Probable Number (MPN) Technique was used for the water Analysis (APHA, 1998). Ten milliliter of single strength lactose broth was transferred into two test tubes and 10mls of double strength lactose broth (Biotec, United Kingdom) was transferred into the remaining column. Durham tubes (Pyrex) were put into the tubes and sterilized by autoclaving. 10m1 of the sample was inoculated into the tubes with double strength lactose broth, and 0.1m1 of the sample into the next column. The test tubes were incubated at 37°C for 24h for the estimation of total coliforms and at 44.5°C in a water bath for faecal coliforms for 48h. Acid production was determined by colour change of broth from reddish purple to yellow and gas production was checked by gas formation in the Durham tubes. The MPN was then estimated from the MPN table for three tube test.

CONFIRMED TEST

A loopful of culture from a positive tube from the presumptive test was transferred into a tube of Brilliant Green lactose Bile (BGLB) broth (Oxoid) with Durham tubes. The tubes were incubated at 37°C for 24h for total coliforms and at 44.5°C for 48h for faecal coliforms and observed for gas production.

COMPLETED TEST

A loopful of broth from a positive tube was streaked onto Eosin Methylene Blue (EMB) agar plate for pure colonies. The plates were incubated at 37°C for 24h. Metallic sheen colonies on EMB agar, were presumptively considered to be *Escherichia coli* and their identities were confirmed using morphological and biochemical tests. For faecal coliforms, colonies with green metallic sheen were gram stained and the indole, methyl red, Voges Proskauer and Citrate utilization (IMViC) test was carried out on Nutrient agar stock cultures and used to identify the colony as *E. coli*. The MPN per 100ml water was calculated using completed test.

IDENTIFICATION OF BACTERIAL ISOLATES

Stock culture of the isolates with different cultural characteristics was made on nutrient agar slants. Gram staining was used to check for morphology and biochemical tests were performed to aid in identification. Various tests performed and used in probable identification of isolates included the Gram staining procedure, Oxidase test, Motility test, catalase test, Coagulase test, Indole test, Methyl red test, Voges-Proskauer and Citrate utilization test as described by Cheesbrough (2000).

DATA ANALYSIS

Data were subjected to analysis of variance (ANOVA) using Statistical package for social sciences (SPSS) version 16.

RESULT

Table 1 shows the Total Heterotrophic Bacteria Count (THBC) obtained in this study. Sample **A** had a Mean Heterotrophic Bacteria Count of 1.0×10 while Sample **B** had 2.2×10 .

Sample **C** had a Mean THBC of 1.3×10 and Sample **D** 2.3×10 . Again, Sample **E** had 2.3×10 while F had 4.7×10 . Sample **G** had a Mean of THBC of 0.9×10 while Sample **H** had 0.5×10 .

Sample **I** had 0.9×10 and Sample **J** 0.2×10 . There was no significant difference ($P > 0.05$) in the ANOVA.

Samples **A, B, C, E, H** and **I** had a mean of Total Coliform Bacteria of 5/100ml, Samples **E** and **G** had 2/100ml and **D** and **J** had 0/100ml respectively. There was no significant difference ($P > 0.05$) in the ANOVA as shown below in table 1. The Mean of all the samples for Faecal Coliform Count was zero. There was no significant difference ($P > 0.05$) in the ANOVA (Table 1).

Table 1: The Total Heterotrophic Bacteria Count (THBC) and Mean of Total Coliform Bacteria (MPN/100ml) IN SACHET Water Samples

Water sample	Total Heterotrophic Bacteria (THBC) (Cfu/ml)		Mean Total Coliform Bacteria of Water Samples (MPN/100ml)	
	Ranges	Mean	Ranges	Mean
A	$0.9 \times 10 - 1.0 \times 10$	1.0×10	0 - 9	5
B	$2.1 \times 10 - 2.2 \times 10$	2.2×10	0 - 9	5
C	$0.7 \times 10 - 1.9 \times 10$	1.3×10	0 - 9	5
D	$0.9 \times 10 - 3.6 \times 10$	2.3×10	0 - 0	0
E	$1.4 \times 10 - 3.2 \times 10$	2.3×10	0 - 9	5
F	$1.2 \times 10 - 8.2 \times 10$	4.7×10	0 - 4	2
G	$0.4 \times 10 - 1.3 \times 10$	0.9×10	0 - 4	2
H	$0.1 \times 10 - 0.8 \times 10$	0.5×10	0 - 9	5
I	$0.8 \times 10 - 0.9 \times 10$	0.9×10	0 - 9	5
J	$0.1 \times 10 - 0.2 \times 10$	0.2×10	0 - 0	0

Table 2 below showed the bacteria isolated from the sachet water Samples. *Staphylococcus* spp has the highest occurrence of 32% in the studied water samples,

followed by *Pseudomonas* spp 23%, *Klebsiella* spp 20%, *Proteus* spp 15% and *Enterobacter* spp 10%.

Table 2: Bacteria isolated from the sachet water Samples

Sachet water	% of water with Bacteria	A	B	C	D	E	F	G	H	I	J
<i>Enterobacter</i> spp	10	-	+	-	-	-	+	-	+	-	-
<i>Staphylococcus</i> spp	32	+	-	+	+	+	-	-	+	-	-
<i>Proteus</i> spp	15	-	-	+	+	+	-	-	-	+	-
<i>Klebsiella</i> spp	20	+	-	-	-	+	+	-	-	-	-
<i>Pseudomonas</i> spp	23	-	+	+	-	-	+	+	+	-	+

DISCUSSION

The result of this study showed the presence of bacteria in the water samples studied. This is similar to the studies by Adekunle *et al.*, (2004) in Ibadan, Ezeugwunne *et al.*, (2009) in Nnewi Oladipo *et al.*, (2009) in Ogbomoso, all in Nigeria which showed the presence of some bacteria in sachet water and Addo *et al.* (2009) in the study of bacteriological quality of sachet water produced and sold

in Teshie-Nungua suburbs of Accra, Ghana which detected faecal coliforms and *Escherichia coli* which were attributed to poor treatment and handling methods in some sachet water producing industries. This could be as a result of poor environmental conditions, poor handling by distributors and sellers or insufficient sterilization of the sachets use in packaging the water or contamination with bacteria of the vending machine use in packaging

sachet water or the duration of the sachet water. The international standards for drinking water states that

portable water should not contain 100cells of heterotrophic bacteria per 100ml of water (WHO, 1984). Five (5) brands (A, G, H, I, J) out of 10 of these sachets water studied met up with this standard and this could be as a result of proper location of the water source and effective treatment which the water has received, therefore the public who drink these water could be free from water borne diseases. However Egwari *et al.* (2005) in Lagos, southwest Nigeria and Ekwunife *et al.*, (2010) at Awka, Southeast Nigeria in their bacteriology study of sachet water found no enteric pathogens and *Entamoeba coli* in the water studied.

The multiple tube test study showed the presence of coliform bacteria which is an indicator bacteria that is used to evaluate the quality of drinking water and the absence of faecal coliform which indicate the contact of water with sewage or inadequate treatment or post treatment contamination. World Health Organization (WHO) standards for treated water says that no sample of 100ml should contain more than three coliform organisms and *Escherichia coli* should not be detected in any sample of 100ml (WHO,1984). Egwari, *et al.*, (2005) in their study on bacteriology of sachet water sold in Lagos reported that organisms contained in the wastewater were inevitably the source of contaminants on the sachet surface. The water vendors and their patrons contributed to the overall contamination of hawked sachet water in lagos.

Again, the water samples studied in this work met up with this standard which could be attributed to the effective water treatment and proper location of water source away from sewage tanks. In order to protect public health and ensure that water is safe for public use, any water intended for drinking treated or untreated, piped or unpiped must meet certain microbiological standard. A violation of set standards warrants treatment of the present source or the need for an alternative water supply.

The result of this study also showed the presence of some micro organisms. This is similar to the studies by Adekunle *et al.*, (2004) and Obire *et al.*, (2009) in the assessment of implications of sachet water. Bacteria isolated from sachet water in this study include *Enterobacter* specie, *Staphylococcus* specie, *Proteus* specie, *Klebsiella* specie and *Pseudomonas* specie. The presence of *Enterobacter* and *Proteus* species in water samples suggests that these organisms could originate from burst pipe along distribution lines of drinking water or unhygienic handling of water right from the treatment plant used in production of such water (Edema *et al.*, 2001) while the presence *Staphylococcus* specie could

be as a result of ubiquitous nature of the organism or poor staff handling during water processing (Edema *et al.*, 2001). *Pseudomonas* spp. whose presence is of significant value in determining the extent of water pollution and *Klebsiella* spp. may be as a result from contaminated vending machine in United States (Oladipo *et al.*, 2009). Earlier investigation conducted on safety of drinking water has shown that water on the market is of good microbiological quality while the quality of some factory bagged sachet and hand filled polythene bagged drinking water was noted to be doubtful (Obiri- Danso *et al.*, 2003).

Egwari, *et al.*, (2005) in their study on bacteriology of sachet water sold in Lagos reported that enteric pathogens and *Escherichia coli* were not isolated from any samples and brands of sachet water but formed significant part of the isolates on the sachet surfaces of samples collected from the cooling receptacles (pail, wheelbarrow and refrigerator). Similar species of bacteria were isolated from wastewater and surface of the sachets with the wastewater containing a significant higher numbers of bacteria while Adegoke *et al.*, (2011) reported the presence of *Proteus* sp, *Klebsiella* sp, *Enterobacter* sp, *Pseudomonas* sp, *Staphylococcus* sp and *Escherichia coli* in borehole water at Oyigbo, Rivers state suggesting that those borehole water did not meet WHO standard.

CONCLUSION

The bacteriological examination of Sachet Water sold in Aba Town suggested that 5 brands out of 10 brands studied met up with WHO Standards in the areas of Faecal contamination, the number of Heterotrophic Bacteria Count and the number of different or individual bacteria allowed in drinking water. It was also shown that most of the brands of the Sachet Water had bacterial growth, hence, the word "Pure Water "used instead of Sachet Water is practically incorrect. This may be as result of poor handling. Educating the water handlers' i.e. sellers, distributors and producers could be of help in reducing or eradicating incidences of water transmitted infections.

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