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Bacteriological Examination and Antibiotic Resistance Profile of Bacteria Isolated from Bottled Water Sold in Amai, Delta State.

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ABSTRACT

This study was carried out to investigate the microbiological quality as well as the antibiotic resistance profile of bacterial species in some bottled water sold in Ukwuani Local Government Area of Delta State. The presence of heterotrophic plate count from six (6) commercially available bottled water were examined using standard pour plate and spread plate methods, while the antibiotic resistance was investigated using disc diffusion method. The results obtained indicated that 16.7% and 83.3% of the samples showed heterotrophic plate (HPC) count within the range of less than 1(CFU/ml) and 1- 250 (CFU/ml), respectively, and 0% sample showed greater than 250 (CFU/ml) in heterotrophic plate counts. The results also showed that 83.3% klebsiella, 83.3% *Staphylococcus aureus*, 50% Salmonella, 50% Serratia, 66.7% *Escherichia coli* and 16.7% Streptococcus, were isolated from the water samples. All the bacterial isolates were resistant to the antibiotics tested. Since some bacterial species have developed resistance and are still developing resistance against certain antibiotics which were once known to be active against such bacterial species, it is therefore recommended that good treatment techniques that would eliminate all forms of microorganisms that could cause water related diseases be used to treat bottled water before distributing to the consumer populations.

Keywords: Bottled Water, Resistance, Heterotropic Plate Count, Antibiotics, Isolated, Microorganisms.

INTRODUCTION

The recent introduction of sachet and bottled water to consumers was aimed at providing safe hygienic and affordable 'instant' drinking water to the public and to prevent or control the magnitude of water related infections in Nigeria. Although this is a laudable idea, current trends however seems to suggest that sachet and bottled water could be a route of

transmission of enteric pathogens (Kwaye-Nuako *et al.*, 2007). Sachet and bottled water is water (from either tap, well, e.t.c), that has been treated, processed and sealed in a package (either polyethylene bags, plastic or glass bottles), distributed for sale under sealed food grade material or other appropriate containers and intended for human consumption (Food and Drug Administration, 2002). The intake of unwholesome water could have devastating effects on our health as unsafe drinking water is a key determinant of many microbial diseases with serious complications in immunocompromised as well as healthy individuals (WHO, 2008).

Bottled and sachet water consumption has grown steadily in both developed and developing nations worldwide for the past 30 years. It is the most dynamic sector of all the food and beverages industry. Consumption in the world increases by an average of 12% each year in spite of its high price compared to tap water. Consumers may have various reasons for purchasing bottled and sachet drinking water such as taste, fashion or convenience, but for many consumers, safety and potential health benefits are important considerations because they believe that bottled and sachet water is safer than tap water (WHO, 2008).

The truth about Nigerian water situation is that packaged water has come to stay and demand will continue to increase, thereby putting the fate of the consumers in the hands of the packaged water producers and dealers. Although, bottled water should have a shelf life of 30 days unopened, most bottled water companies' label showed that their water is valid for 1 to 2 years (Kendall, 2007). Safe drinking water is very scarce and hence, the need for processing, treating and proper packaging of water before consumption (Anaekwe, 2012).

Several studies on the microbial quality of bottled and sachet water have shown violations of international standards. The most widely used indicator for microbial water contaminant is the coliforms group of microorganisms. Coliforms are used as indicator of water contamination because many of them inhibit the intestinal tract of humans and other animals in large number, thus, their presence in water indicates fecal contamination. Coliforms are gram negative, facultative aerobic rod shaped bacteria that ferment lactose with gas formation in 24hrs at 37°C.
Pathogenic organisms like *Shigella spp., Leptospira spp., Salmonella paratyphi B, Vibrio, Staphylococcus aureus, Pseudomonas aeruginosa* and *Aeromonas spp.* can be isolated from

drinking water samples (Rajendran *et al.*, 2006). Epidemics of cholera have been reported from different parts of India. The outbreak was caused by *Vibrio cholera* isolated from municipal taps and wells (Sur *et al.*, 2006).

A report showed that of the 27 sachet water samples studied in Accra, Ghana; 77% contained parasitic organisms (Kwaye-Nuako *et al.*, 2007). The organisms isolated included parasitic stages of *Microsporidium spp., Cryptosporidium spp., Sarcocystis spp. and Cydospora gayenthensis.* Another study showed that *Staphylococcus aureus, Enterobacter fecalis, Moraxella catarrhalis, Shigella spp. and Serratia marcescens* were isolated from sachet water samples sold in Ghana (Tagoe *et al.*, 2011). Levels of contamination in bottled and sachet water are seldom high enough to cause acute health effects. Examples of acute health effects are nausea, lung irritation, skin rash, vomiting, dizziness diarrhea, gastroenteritis and even death (Sandra, 1996). Other infections caused by microbial pathogens are Salmonellosis, Dysentery, Gastroeneritis, Shigellosis, Hepatitis, Giardiasis, Abdominal cramps etc.

The need to access the quality sachet and bottled water has become imperative, because consuming water with pathogenic organisms poses a great risk on the individuals and has a direct effect on the health of its consumers. Majority of sachet and bottled water are produced under questionable hygienic environment, without approval of the Government and does not meet standards (Addo *et al.*, 2009). Low-income populations are particularly at risk of such diseases because of the lack of safe water and sanitation (Classen *et al.*, 2006).

The emergence of bacteria resistance to most of the commonly used antibiotics or drugs is of considerable medical significance, because of the public health implications (Khan and Malik, 2007). The occurrence of multiple antibiotic resistance (MAR) bacteria is a well known phenomenon, and many investigators believe that these drug resistant organisms have become more common recently due to the extensive use of antibiotics in medicine and agriculture throughout the world. Concern about this situation has also become more common, since the antibacterial value of drug is threatened seriously by the increased prevalence of

resistant bacteria (Khan and Malik, 2007).

This concern is particularly relevant in the light of the discovery that resistant characteristics can be transferred to non-resistant recipient genes via water borne bacteria carrying transmissible R-factors. In environmental settings, polluted by human or animal waste or both high frequencies of MAR, bacteria phenotypes exist in the coliforms and fecal coliforms population. These environments include surface water receiving run-off from lands occupied by livestock, estuaries and contaminated water supply (Kelch and Lee, 1978).

When the antibiotic resistance bacteria are introduced into water through fecal contamination, its emerging disease will hardly respond to treatment. A study on 60 packaged water, 60 tap water and 180 well water samples, showed that enteropathogenic bacteria isolated included *Escherichia coli, Klebsiella species, Salmonella species, Shigella species* and *Vibrio species* and their antibiotic resistance was tested (Garba, 2007). It was observed that *E. coli* was resistance to Amoxicillin while *Salmonella sp.* was resistant to Septin and Perfloxacin.

In the rural areas of many Countries, especially Africa, portable water has become a scarce commodity as only a small portion of the populace has access to treated water. Majority of the sachet and bottled water are produced under questionable hygienic environment without the approval of the government, and in addition they serve as sources of antibiotic resistant microbes. All these underscore the purpose of this study in Amai, a rural community in Delta State. Therefore, this study is aimed at determining the bacteriological quality of the water samples sold in Amai and evaluating the antibiotic resistance of bacterial isolates from the water samples.

It is expected that the isolation of microorganisms in the water samples and their antibiotic resistant pattern will be useful for treatment of water borne diseases. The result obtained at the end of the experiment will also add to existing knowledge on the health impact of consuming commercially available contaminated water.

METHODOLOGY

This study was carried out in Amai viilage in Ukwani Local Government Area of Delta state, Nigeria. A total of 6 bottled water samples (ranging from 60- 75cl) sold within Ukwani Local Government Area of Delta state, were bought from retail shops in the Local Government Area. The samples were carried into the

laboratory at Novena University, Ogume for microbiological analysis.

Sterilization

Glass wares like petri dishes, test tubes, Durham tubes, pipettes, conical flasks, were sterilized in oven (Genlab limited, UK), wrapped in foil paper and sterilized at 160°C for 1 hour, while all media were sterilized in the autoclave (Equitron, China) at 121°C for 15 minutes. The work area was cleaned with 70% ethanol and the wire loops were sterilized by flaming in a Bunsen burner until it became red hot. All analysis were carried out under aseptic conditions.

Inoculation and Incubation

Serial dilution was done using peptone water for dilution power of up to 10^{-3}, in accordance with the method of Garba *et al.* (2007). 1ml of sample from each dilution factor was inoculated into Nutrient agar (Fluka Biochemical, Germany), MacConkey agar (Fluka Biochemical, Germany)- to determine the coliforms count and Saboraud dextrose agar (Fluka Biochemical, Germany) (for fungal count) using the pour plate method for enumeration of microbes. The culture plates were incubated at 37°C for 24 hrs.

Isolation and Enumeration

Bacterial Count: viable bacteria count was carried out. Plate count was restricted to 30-300 colonies, and plates with more than 300 colonies were designated as too numerous to count (TNTC), and plates containing less than 30 colonies were designed as too few to count (TFTC). The number of cells per ml and number per 100ml were obtained using the equations below in accordance with the method of Acharjee (2011).

$$Number\ of\ cells\ per\ ml = No.of\ Colony \times \frac{dilution\ factor}{volume\ of\ sample}$$

$$Number\ of\ cells\ per\ 100ml = No.of\ Cells\ per\ ml \times 100$$

A sterile wire loop was used to pick the isolate from the plates and streaked on freshly prepared agar plates to obtain discrete colonies. On the other hand, fungal colonies were counted macroscopically, and characterized after 72 hours.

Identification of Microorganism

Identification of bacterial isolates was based on growth pattern, colony characteristics, colour on agar and different biochemical test such as gram stain, catalase test, coagulase

test, oxidase test, and sugar fermentation (Lactose, Mannitol and Glucose).

RESULTS

The outcome of microbiological analysis performed on samples from bottled water of different brand collected from retail shops in Ukwani Local Government Area, Delta State, are as follows.

The total microbial count of bacteria per 100ml is shown in Table 1. The table represents the heterotrophic plate count (HPC) in the water samples. A total of 6 water samples showed HPC within the range of 1 – 250 CFU/100ml, according to the table; sample B contained the highest amount of bacteria colony per 100ml, while sample F contained the least. None of the samples were totally free from coliforms.

Table 2 shows the heterotrophic plate count of the water sample in the ranges of < 1, 1 – 250 and > 1 CFU/ml. No sample had HPC value of more than 250 CFU/ml, 16.7% of the sample had HPC value of less than 1 CFU/ml and 83.3% of the sample had HPC value of between 1 and 250 CFU/ml.

Table 1: Heterotrophic Plate Count of Bacteria Showing CFU/100ml

Sample	Count(CFU/100ml)
A	149
B	217
C	172
D	183
E	157
F	32
Total	**910**

Table 2: Range of Heterotrophic Plate Count (HPC) in Bottled and Sachet Water

HPC	No. of Positive Sample (%)
HPC (CFU/ml) < 1	16.7
1 – 250	83.3
>250	0

Table 3 showed the six bacteria that were isolated from the analyzed samples and listed as assumed organisms. The probable identification of the microorganisms showed *Klebsiella* sp., *Staphylococcus aureus*, *Salmonella* sp., *Serratia marcescens*, *Escherichia coli and Streptococcus*. The organisms were categorized based on Gram stain, catalase, coagulase, oxidase, sugar fermentation, acid production and gas production.

Table 3: Summary of Biochemical Tests of the Organisms Isolated

Suspected Organism	Gram Stain	Catalase	Coagulase	Oxidase	Indole	Lactose	Glucose	Methyl Red	Mannitol	Gas Production
Klebsiella sp.	-	+	-	-	-	+	+	-		+
Staphylococcus aureus	+	+	+	-	-	-	+	-	+	+
Salmonella sp.	-	+	+	-	-	-	+	+	+	+
Serratia marcescens	-	+	+	-	-	+	+	-	+	-
Escherichia coli	-	+	-	-	+	+	+	+		+
Streptococcus sp.	+	-	-	-	+	+	-			

Table 4 as seen below indicates the percentage of the isolated organisms in the different water samples.

Table 4: Percentage of Bacterial Isolates in the Water Samples

Bacteria Group	Percentage (%)
Klebsiella	83.3
Staphylococcus	83.3
Salmonella	50
Serratia	50
Escherichia	66.7
Streptococcus	16.7

Table 5: Bacterial Species Isolated in Different Bottled Water Samples

Sample/Org.	*Klebsiella*	*Staphylococcus*	*Salmonella*	*Serratia*	*Escherichia*	*Streptococcus*
A	+	+	+	-	-	-
B	-	+	+	-	-	-
C	+	+	-	+	+	-
D	+	+	-	+	+	-
E	+	+	-	+	+	+
F	+	-	+	-	+	-

+: Present, -: Absent

The bacterial antibiotic resistance profile is shown in the Table 6 below. It was observed that *E. coli, Klebsiella* sp. *and Serratia marscecens* were each resistant to Ciprofloxacin, Perfloxacin and Ofloxacin.

Table 6: Bacterial Antibiotic Resistance Profile

	Ciprofloxacin	**Perfloxacin**	**Ofloxacin**
Escherichia coli	R	R	R
Klebsiella sp.	R	R	R
Serratia marscecens	R	R	R

R = Resistant
S = Susceptible

DISCUSSION

The high value of heterotrophic plate count (HPC) obtained in this study indicates high level of contamination in the water sample, and consequently, poor quality of the commercially available packaged water in the Local Government Area. The result of the study agrees with the report that detected HPC in 92% of the bottled water samples analyzed (Kassenga, 2007). Lower results were obtained in a study and it was reported that the percentage of bottled water samples that had unacceptable HPC at 37°C constituted 29.3%, and 8%, respectively (El-Batouti, 2002; Richards *et al.*, 1992). It was added that carbonated bottled water had a lower HPC which may be attributed to the use of carbonation as a final step of disinfection; a process which can lower the pH of the product and significantly reduce the bacterial load (Warburton, 2000). It was also reported that 85% of the examined bottled water samples collected from Egypt had uncountable HPC (Abdel and Hassan, 2000). Higher results were obtained in Nigeria, where it was found that 34.5% and 53.5% of the examined samples, respectively had counts that exceeded 104 CFU/ml (Health Canada, 2002). Much higher counts

(104 to 106 CFU/ml) were also reported in four Egyptian brands of bottled water (Abdel and Hassan, 2000). The results on the bacteria isolated from the water samples indicate the presence of *Klebsiella* sp. (83.3%), *Staphylococcus aureus* (83.3%), *Escherichia coli* (66.7%), *Streptococcus* sp. (16.7%), *Serratia marcescens* (50%) *and Salmonella* sp. (50%).

Klebsiella sp. and *Staphylococcus aureus* were the most isolated, followed by *Escherichia coli*. This results shows that some water samples are more contaminated than others. This could be due to environmental conditions and processing. Heterotrophic plate count is a potential indicator of overall sanitation in bottled and sachet water. It may be harmless in them, but in some cases may indicate presence of infectious bacteria. High number of HPC of bacteria in bottled might come from the non sterile plastic bottles that arrive at the plant and may have been transported without caps, thus the interiors are exposed to airborne contamination and the presence of foreign matter or contaminated equipment during production. However high HPC values may also indicate poor GMP during the processing of the water and it may be used to determine the suitability of water for use in the manufacture of food and drinks. In order to minimize spoilage, the number must be low.

The antibiotic resistance pattern obtained in this work agrees with the report on a study carried out on 60 packaged water, 60 tap water and 180 well water samples, which showed that the enteropathogenic bacteria isolated included *Escherichia coli, Klebsiella* species, *Salmonella* species, *Shigella species* and *Vibrio* species and their antibiotic resistance was tested. It was observed that *E. coli* was resistance to Amoxicillin, *Salmonella sp*. resistant to Septin and Perfloxacin (Garba, 2007).

The presence of indicator organisms indicates that water is contaminated by potentially dangerous fecal matter and hence their absence denotes in general the water safety. Although, coliform organisms may not always be directly related to presence of fecal contamination or pathogens in drinking water, the coliform is still useful for monitoring the microbial quality of drinking water. Usually only *E. coli* is considered as a specific and reliable indicator of fecal pollution of water. They are indicated in

gastroenteritis, typhoid fevers and diarrhea.

Investigations on *Staphylococcus aureus* are recommended to indicate poor hygiene practices during the bottling and packaging process as staphylococci are part of the normal skin flora. In this research work, *S. aureus* was detected from 83.3% of the samples. This high value may be associated with contamination not only from the containers, but also from the physical surroundings and the people who came in contact with the bottling and packaging process.

Bottled and sachet water is usually disinfected to remove harmful organisms, but is not intended to sterilize it, so bacteria are found in most bottled and sachet water in minimal permissible amounts. To maintain the purity of packaged water, it is recommended that bottled water should be refrigerated once open preferably once bought. Checking the manufacturing and best-before-date on the bottle or sachet determines how fresh the product is. Health Canada study of bottled water kept at room temperature for 30 days showed a substantial increase in the bacterial count, especially HPC (Health Canada, 2002).

CONCLUSION

Bottled and sachet water market has steadily increased over the last decade and a number of people now prefer to consume them for a number of reasons. They may not like the taste or color but they continue to consume them in need. On the other hand, some people are worried about their health and perceive packaged water as safer and healthier alternative to water supplied by local authorities, although not all packaged water have the same qualities. Also, proper sanitation practices should be implemented during production of bottled water. Furthermore, improved monitoring of water and frequent application of chlorine and other water treatment agent should be adopted.

It is therefore recommended that processing practice should be improved, the choice of packaging materials should be considered. It should also be noted that indiscriminate use of antibiotic drugs should be avoided, as it is a major cause of bacterial resistance in microorganisms.

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