JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2008. Vol. **2**(1), p. 97-102

Microbial Analysis of Bottled Water Samples Sold Commercially in Umuahia Metropolis in Abia State, Nigeria.

Uchechi N. Ekwenye* and Chidinma U. Ehilegbu

Department of Microbiology, Michael Okpara University of Agriculture, Umudike P.M.B. 7267, Umuahia, Abia State, Nigeria.

(Received: 14 February 2008; accepted: 30 March 2008)

Bottled water samples from Umuahia Metropolis were analysed for their microbial and physiochemical properties. Although some properties like pH, temperature and others are known to contribute to the growth of these microorganisms. The absence of coliform organisms or pathogenic microorganisms such as *Clostridium perfringes* and *E. coli* in these samples showed that the water was safe for drinking. Some of the organisms observed were *Streptococcus spp, Proteus spp,* and *Pseudomonas spp* and some physiochemical properties such as turbidity, total solids, dissolved oxygen, sulphate, potassium, hardness as well as colour, odour, taste and temperature were also determined.

Key words: Physiochemical properties, *Streptococcus* spp, *Proteus* spp, *Pseudomonas* spp, Bottled water.

Man must have abundant supply of water free from pathogenic microorganisms and deleterious substances in order to protect his health and ensure his well being (Richard *et al*., 2000). Water which contains odour, colour, taste or any organism is said to be polluted. Water may contain some pathogenic organism, which could cause disease to consumers and may also be harmful to aquatic life. Some water-borne and water associated diseases include schistomiasis (biharziaris), guinea worm, giardiasis, cholera, to mention but a few. Most importantly water transmitted diseases often lead to gastroenteritis in young children, diarrhea, dysentery and enteric fever, which are typical of pathogenic organisms like *E. coli* (Okafor, 1985).

* To whom all correspondence should be addressed. E-mail: ekwenyeuchechi@yahoo.com.

Most of the water sources are borehole, wells and springs from pit latrine, septic tanks and other waste disposal site. Polluted water can travel long distance underground when condition allows (Davis *et al*., 1993).

To ensure microbiologically safe water supplies require an approach, which eliminates pathogenic microorganism. The menace caused by water borne pathogens in recent times has given much concern to public health workers especially considering the huge losses both in human and material resources emanating from the exploits.

Regular sampling and analysis of water provides data on the quality of raw water, the microbial load and the efficiency of treatment. The use of indicator organism is based on the assumption that if they are present, the pathogens may also be present, but if they are absent, then the water is suitable for consumption. In most cases, the indicators are present when contamination has occurred and is usually in higher

number than the pathogens. The principal indicators are the coliforms, faecal coliforms, enterococci, *Clostridium perfringes* and total aerobic bacteria (Richard *et al.,* 2000). Moreover packaged drinking water is rarely completely free of microorganism. Typically the autochthonous flora present at sources are of little concern to a healthy population. However, the predominant bacteria include *Pseudomonas,* some of which are known to be opportunists (Rosenbery, 2003). To reduce or kill pathogenic microorganisms and make the water safe for drinking, several methods can be implemented such as boiling at 100^0C though most microorganisms have been tested to be thermophiles (surviving in high temperature) but a reasonable manner high temperature inhibits the growth of microorganisms and thereby making them inactive. Other methods can be implemented such as filtration, chlorination, alkalinity reduction and coagulation and flocculation.

Water could be tested to be free of odour, taste and particles but could still be said to be unsafe for drinking. The drastic increase in water related diseases such as typhoid, dysentery, cholera, diarrhea, hepatitis in our immediate environment has led to the treatment of water to ensure the decrease in the outbreak of these diseases.

Non-availability and inadequate distribution factor and also low standard of living have led to the reoccurrence of these epidemics in our society. The focus of this research work is to examine some samples of portable water to know the bacteriological status and physiochemical property of these samples of portable water, which will help us to guide against water related diseases.

MATERIAL AND METHODS

Sources of materials

Eight samples of bottled water which was sold commercially in Umuahia were obtained and analysed. They are designated or coded as:

Sample 1	=	Lily
Sample 2	=	Nason
Sample 3	=	Ragolis
Sample 4	=	Eva
Sample 5	=	Uzzy
Sample 6	=	Formosa
Sample 7	=	Gossy,
Sample 8	=	Polar

Methods of Laboratory analysis

Preparation of media

MacConkey agar

The media MacConkey agar (MA) and Nutrient agar (NA) were prepared according to the manufacturer's instructions.

The medium was used in the test for possible coliforms. It was prepared by dispersing 52gms of the dry powder in 800ml of water. The mixture was heated to melt and made up to 1 litre with distilled water. The pH was measured to conform to the range of 6.8-7.0 and then sterilized by autoclaving at 121°C in 15 psi pressure for 15 minutes.

Nutrient agar

Twenty eight gms of the dehydrated powder was dispersed in some of distilled water in a pyrex glass flask. The medium in the flask was heated in a water bath until the agar dissolved. Then it was made up to 1 litre with distilled water, the pH was checked to be within the appropriate range of 6.0-7.0 before sterilizing in the autoclave at 121°C in 15 psi pressure for 15 minutes. After sterilization just before the medium was poured into plates, some fungusol centrifugal power was added to control possible growth of fungi.

Bacteria enumeration

The spread plate technique described by Pelczar and Chan (1977) was used. Inoculum of 0.1ml was transferred to sterile solid agar (NA) in a petridish. A sterile glass hockey was used to spread the inoculum all over the surface of the medium. The inoculation was done in triplicate and the plates were incubated at 37°C upside down in triplicate in an incubator. The plates were observed for growth and subsequently counted using a Gallenkamp colony counter. A means of the triplicate count was determined and multiplied with the dilution factor to obtain the counts which were expressed as colony forming units per milliliter of the water sample.

Determination of bacterial flora of the water samples

The methods described by both Pelczar and Chan (1977) and Fawole and Oso (1988) were followed –following a 48hours spread plate culture of the water samples. The water samples were gently shaken and 0.lml transferred into a sterile plate and 1ml in another plate and labeled

appropriately. 0.1ml of each sample was added to 9.9ml sterile water and mixed properly to give 10^{-2} dilution. 1ml of the 10^{-2} dilution was transferred to a sterile petri dish and labeled appropriately. The molten agar was poured into plates containing the water samples and incubated at 37°C for 48 hours and the colonies counted. (Pelczar and Chan, 1977; Fawole and Oso, 1988). The culture was examined for the presence of bacteria and transferred to sterile agar plates (NA and MA). The plates were incubated for 48 hours and examined for uniformity which was taken as indicative of purity. From the resulting pure culture, each bacterium seen was transferred to sterile agar slants in MacCartney bottles as isolates.

Each bacteria isolated from the water samples was subjected to a four step analysis to establish its identity. The steps involved cultural examination, microscopic examination, biochemical reactions and carbohydrates utilization tests.

Cultural examination

Pure cultures of the isolates were examined closely to determine the colonial characteristics of each of the isolates. The colonies were observed for their shapes, size, elevation, form, consistency, colour and general nature.

Microscopic examination

From colonies in the pure cultures, the bacterial isolates were examined under the microscope. Gram staining was carried out and during the test, the arrangements and shapes of the bacterial cells were observed and recorded. The other tests carried out included test for spores (sporestain), test for flagella (flagella stain), test for motility.

Biochemical tests

Biochemical tests carried out included catalase H25, urease, indole, oxidase and coagulase production, nitrate reduction, methyl red, voges proskauer and carbohydrate utilization tests following the methods described by Pelazar and Chan (1977) and Fawole and Oso (1988).

Coliform test

Test for the presence of coliforms in water samples was done at various level namely: presumptive and confirmatory. During the presumptive tests, the multiple tube fermentation technique (Pelczar and Chan, 1977) was used to determine the Most Probable Number (MPN) of the coliform bacteria present in the water sample.

Measurement of water quality parameters

pH

The pH was taken with the use of pH meter. The meter was dipped into the water samples and the values were read off on the digital display board.

Temperature

0-50°C mercury bulb thermometer was used to determine the temperature of bottle H_2O. The mercury bulb was dipped into the water and allowed to stabilize. After two minutes, the temperature was read off and recorded in °C. Care was taken to prevent the bulb from touching either the side or bottom of the beaker (American Public Health Association, 1985).

Turbidity

Turbidity was measured by placing 25ml of water sample in a cuvette and read at 425nm using a spectrophotometer and expressed as NTU (Normal Turbidity Unit).

Conductivity

Conductivity meter was used; the conductivity probe was inserted into the water sample and the value was read off as given.

Dissolved oxygen

The winkler bottle (118-120ml) was filled with water sample, 2ml of Mno_4 solution and 2ml of alkaline iodine solution were added beneath the surface using a dropper or pipette, in such a way that no air bubbles were trapped beneath it. The mixture was shaken vigorously and allowed to dissolve the precipitate. It was then titrated with 0.02M thiosulphate solution, 3 drops of starch solution was added when the light yellow colour was really faint. A blue colour was formed which was titrated to a colourless solution.

Total hardness

Total hardness was determined by ethylenediamine tetracetic acid (EDTA) titration using Erichrome Black T as indicator. To 50mls of water sample in 100ml beaker were added 10ml of buffer (NH_3) solution and two drops of indicator. The solution was titrated with 0.02M EDTA. Titration stopped as colour changed from wine red to blue.

Calculation

Total hardness/Volume of sample = ml of

0.02M EDTA × 1000 (American Public Health Association, 1985).

Total Solid

The solid content was determined by the gravimetric method. A measured volume of each water sample was dispensed into a weight moisture can. The can and its water content were placed in a steam bath and the water evaporated to dryness. Then the can was further dried in the oven at 105°C for 60 seconds. After cooling in a desiccator, it was reweighed. By difference, the weight of solids obtained was determined and expressed in mg/l, which is given by the expression below:

Total solids (mg/l) = $1000 \times W_2 - W_1 \times 1000$

where

V = Volume of water analysed

W_1 = Weight of empty moisture can

W_2 = Weight of can + solid.

Acidity

Direct alkaline titration methods described variously by Pearson (1976) was used. Ten mililitres of each test water sample was dispensed into a clean glass conical flask. Two drops of phenolphthalein indicator solution was added and swirled to mix well. The test water sample was litrated against 0.1N NaoH solution. The total litrable acidity was calculated as shown below:

$$\text{TTA mg/l} = 1000/V \times N \times T$$

where

N = Normality of titrant (NaoH)

V = Volume of water sample

T = Titre value.

RESULTS AND DISCUSSION

The eight samples of different bottled water sold in Umuahia metropolis were observed to be colourless, odourless, tasteless and acceptable. The pH of the samples were between 7.30 and 7.66. The temperature of the samples was 28.5°C and turbidity ranged from 0.14units to 0.17 units (Table 1). The chemical parameters of the water samples are shown in Table 2 and they met the World Health organization (WHO) (1984) standard for portable water. Water samples were cultured and isolated into three plates and their bacterial load reported which has a close range

Table 1. Physical parameters of the water samples

Parameters	1	2	3	4	5	6	7	8
Colour OdourTaste General acceptability	CLS OLSTLS ACB	CLS OLSTLS ACB	CLS OLSTLS ACB	CLS OLSTLS ACB	CLS OLSTLS ACB	CLS OLSTLS ACB	CLS OLSTLS ACB	CLS OLSTLS ACB
pH	7.30	7.58	7.62	7.66	7.50	7.36	7.52	7.62
Temperature	28.5	28.5	28.5	28.5	28.5	28.5	28.5	28.5
Turbidity	0.15	0.16	0.15	0.16	0.14	0.15	0.17	0.14

CLS – colourless OLS – odourless TLS- tasteless ACB – acceptable

Table 2. Chemical parameters of the water samples

Parameter	1	2	3	4	5	6	7	8
TS%	0.02	0.02	0.01	0.02	0.01	0.02	0.02	0.01
TTA%	0.18	0.20	0.15	0.13	0.14	0.19	0.16	0.15
DO (mg/l)	0.93	0.97	1.03	0.97	1.05	0.95	1.03	0.97
Sulphate(mg/l)	24.40	25.60	30.00	25.60	32.50	30.60	26.20	24.80
Potassium(mg/l)	21.00	16.10	18.20	19.50	18.20	14.60	14.80	16.10
Hardness(mg/l)	145.50	135.00	140.00	150.00	128.00	152.00	144.00	142.40

when compared with the World health Organization (1984) standard and thus confirmed the sample as generally acceptable as seen in Table 3. Also the characterization and features of the different isolates were also noted and shown. In Table 5. though some species of microorganisms like *Pseudomonas sp., Staphylococcus sp.* and *Proteus sp.* were present in the various waters in different percentages, *Staphylococcus* and *Pseudomonas* are seen in the same percentage as shown in Table 4 and this attributes to the fact that *Staphylococcus* are said to be normal flora of the human skin, respiratory and gastrointestinal tract and must have contaminated the water during the process of packaging. The *Pseudomonas sp.* which is widely seen in soil, water, plants and animals can also be seen as a heterotrophic flora of water sources. These opportunistic pathogens may sometimes be dangerous to water environment. (WHO, 1985).

Also, microstain reactions such as Gram staining, spore, flagella capsule and motility tests were carried out. Cell arrangement were also noted for the three isolates as well as other biochemical reaction tests as shown in Table 7 and carbohydrate utilization test confirmed the presence of *Staphylococcus sp, Pseudomonas sp* and *Proteus* sp as shown in Table 8.

Coliform test was also conducted and the result revealed the absence of coliform in each of

Table 3. Bacterial load

Samples	1st plate	2nd plate	3rd plate	Means $\times 10^2$
1	3	3	2	2.67×10^2
2	3	4	4	3.33×10^2
3	3	4	3	3.33×10^2
4	3	3	4	3.63×10^2
5	3	3	4	4.00×10^2
6	3	3	3	2.21×10^2
7	3	4	2	3.30×10^2
8	3	3	4	2.92×10^2

Table 4. Occurrence of Bacterial isolates

Samples	*Pseudomonas* spp	*Staphylococcus*	*Proteus* spp
1	-	-	+
2	+	+	-
3	+	+	+
4	-	-	+
5	+	+	+
6	-	-	+
7	-	-	-
8	+	+	-
occurrence	50%	50%	60%

Table 5. Result on microbial flora determination

Features	1st isolate	2nd isolate	3rd isolate
Features	Smooth circular opaque colonies on nutrient agar	Small circular elevated colonies on nutrient agar with slight pigmentation	Large swarming opaque colonies on nutrient agar.

Table 6.

	Microstain	Reactions	
Gram	+	-	-
Spore	-	-	-
Flagella	-	+	+
Capsule	-	-	-
Motility	-	+	+
Cell arrangement	oval cell in group	small short rods	straight small rods

Table 7. Biochemical reaction Tests

Catalase	+	+	+
Oxidase	-	+	-
Indole	+	+	-
Methl red	-	-	+
Voges-proskauer	+	+	+
No_3	+	+	+
H_2S	-	+	+
Urease	-	-	+
Citrate	-	+	-

Table 8. Carbohydrate utilization

	Staphylococcus spp.	*Pseudomonas spp.*	*Proteus spp.*
Glucose	+	+	+
Sucrose	+	+	+
Lactose	+	-	+
Maltose	+	+	+
Mannitol	+	-	-
Xylose	+	-	+

the sample confirming the fact that the water samples were safe for drinking and met the bacteriological quality standard of drinking water according to WHO (1998).

Therefore from the results above, it could be said that any form of contamination could have resulted during the process of packaging and distribution of the water samples.

In conclusion, from the research carried out on bottled water samples sold in Umuahia metropolis it could be concluded that there were microorganisms present on the cultures incubated which were later identify to be non- pathogenic to human health based on the NAFDAC (1998) and WHO (1984) standards for drinking water. Also, the physiochemical properties of these water samples showed that the water is generally safe for consumption based on the standards.

REFERENCES

1. American Public Health Association. Standard methods for the examination of water and waste water. 16th Edition, APHA, Washington 1985.
2. Davis, J. Gravey and Wood, M., Developing and Managing Community Water Supply Oxford. 1993; 9-32
3. Fawole, M.O. and Oso, B.A., Laboratory Manual of Microbiology Spectrum Books Limited, Ibadan 1988.
4. NAFDAC. NAFDAC Story in NAFDAC consumer safety Bulletin, 2003; 10-18.
5. Okafor, N., Aquatic and Water Microbiology. Fourth Dimension publishing Co. Limited. 1985; 3-5.
6. Pearson, The Clinical Analysis of Food. Churchill Livingstone, Edinburgh 1976.
7. Pelczar, M.J and Chan, E.C.S., Laboratory Exercise in Microbiology. McGraw-Hill The. USA 1977.
8. Richard, R.K., Bath C.A and Patel, P.D., Encyclopedia of Food Microbiology., Academic Press Limited, London 2000; **3**: 812-820.
9. Rosenbery, A.S., *Microbiology of Bottled Water Clinical Newsletter* 2003; **23**: 41-44.
10. World Health Organization. Guideline for drinking water quality. Vol. 1. Recommendation WHO Geneva. 1984; 5-6.
11. World Health Organization. Guideline for drinking water quality (Drinking water quality control in small community supplies) WHO, Geneva 1985; **3**: 121.
12. World Health Organization., Guidelines for drinking water quality, Geneva 1998; **3**: 6-18.