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Evaluation of the Bacteriological and Physicochemical Qualities of Some Brands of Bottled Water Sold in Owerri, Imo State

Kingsley Kelechi Onyekachi¹, Joy Nkeiruka Dike-Ndudim¹, Emeka Simon Anikwo^{2*} and Chizaram Winners Ndubueze¹

¹Department of Medical Laboratory Science, Faculty of Health Sciences, Imo State University Owerri, Nigeria. ²Department of Human Physiology, Faculty of Basic Medical Sciences, Imo State University, Owerri, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This work was carried out in order to detect the presence of bacterial agent in the bottled water samples, and to evaluate the physicochemical qualities of these bottled water. Twenty samples selected from four different brands of bottled water sold in Owerri, were examined to determine their bacteriological and physicochemical qualities. Bacteriological analyses using Membrane filtration technique was carried out to determine the heterotrophic bacteria, total coliform and fecal coliform counts. Physicochemical qualities were also determined using standard methods. The heterotrophic bacterial count ranged from 0.00 to 12.00 CFU/ml, total coliform count ranged from 0.00 to 7.50 coliform/100ml. There was absence of fecal coliform in the samples. The pH, Color, Turbidity and Conductivity of the samples ranged from 5.18 to 7.28, 0.00 to 18.00 PCU, 0.55 to 1.62 NTU and 16.00 to 262.00 µs/cm respectively. The Chloride, Iron and Nitrite content of the samples ranged from 16.99 to 27.98 mg/L, 0.01 to 0.07 mg/L Fe, and 0.00 to 0.34 mg/L

*Corresponding author: Email: chizaramwinnersndubueze@gmail.com;

respectively. The bacterial species isolated belong to the genera *Staphylococcus, Proteus, Klebsiella, Bacillus and Escherichia*. Quality wise, most of the water samples met the XYZ standard for bacteriological and physicochemical quality for drinking water with the exception of few. It can be deduced from this study, that none of the bottled water is suitable for drinking with regards to the bacteriological and physicochemical parameters tested. Though some samples passed the bacteriological examination, most of them were acidic and therefore not safe for consumption.

Keywords: Bottled water; bacteriological qualities; membrane filtration; physicochemical qualities; coliforms.

1. INTRODUCTION

Water is an important element of life and environment. Being a universal solvent, it is required for most of life processes. Man, animal and plants need water for their adaptation and survival and it must be readily available to meet these needs. Clean, safe and potable water supplies in urban areas in Nigeria are of great need and importance for the sustainability of life and a healthy economy. Portable water availability is one of the major problems facing the world and approximately one third of drinking water requirement of the world is obtained from surface sources like rivers, streams, dams, lakes and canals [1].

The standard model for delivery of safe drinking water through treated pipe borne water at municipal level is grossly inadequate in most developing countries. The public health significance of water quality cannot be overemphasized. Many infectious diseases are transmitted by water through the fecal-oral route. Diseases contacted through drinking water kill about 5 million children annually and make onesixth of the world population sick [2].

Going by the renewed global commitments toward the Millennium Development Goals (MDGs) marked for 2015, the importance and contribution of locally sourced low-cost alternative drinking water schemes to achieve sustainable access in rural and urban settings of developing nations cannot be overemphasized [3].

A high percentage of households in Nigeria, in both urban and rural areas, do not have access to safe drinking water. It is estimated that at least 50% of the population purchase water daily. As a result of the low access to safe drinking water and the hot weather in Nigeria, potable and mobile packaged water are in high demand [4]. With the very hard and difficult economy in Owerri and other states, bottled water is readily available, but not quite affordable to a vast majority of Nigerian population. Bottled water is sold all around the country and it differs in quality due to different manufacturers and this raises concern about the potability of some of these products. National Agency for Food, Drug Administration and Control (NAFDAC) took it upon itself to rid the country off unclean bottled and sachet water by asking the concerns to register with it, a measure aimed at monitoring the manufacturers' activities [5].

The proliferation of bottled water in Nigeria calls for further investigation into the activities of the producers in order to determine the extent of compliance with the laid down standards. Over the past one decade, the Federal Government of Nigeria has been spending so much on primary health care as many health personnel have expressed their concerns over the high incidence of deadly water-borne diseases such as typhoid fever, diarrhea and measles [6]. For this reason, a few have adopted preventive measures by properly boiling and filtering drinking water in their homes. However, in some other homes that are fairly buoyant, a safer way of avoiding contact with these diseases is by drinking already bottled water. The product was introduced into the Nigerian market in order to provide safe drinking water devoid of waterborne diseases. However, with the proliferation of companies producing bottled water, there is deviation from the intended goal of providing safe drinking water [3].

Water-related diseases are the major cause of mortality and morbidity worldwide. Among these, diarrheal diseases are estimated to cause 1.8 million deaths each year, mostly in developing countries [2].

Improved water supply and proper sanitation can reduce the occurrence of these diseases.

However, outbreaks of water and food-borne diseases still often occur, even in developed countries. In the United States, 76 million cases of food borne illnesses occur every year resulting in 325,000 hospitalizations and 5,000 deaths (CDC, 2005). Pathogenic agents causing these diseases include the enteric bacteria (diarrheagenic Escherichia coli, Salmonella. Shigella and Campylobacter), viruses (norovirus, hepatitis A) and protozoa (Cryptosporidium and Giardia) [7].

Hunter [8] reported that water-borne diseases might account for one third of the intestinal infections world-wide. A total number of 1.2 million water related cases of illness has been reported by Hunter and Syed [9] while Pruss et al. [10] estimated that water, sanitation and hygiene were responsible for 4.0% of all deaths and 5.7% of the total disease burden occurring worldwide. Contamination of water and food with fecal bacteria is and remains, a common and persistent problem, impacting public health, local and national economies [11].

In a society with an increasing demand for safe and potable drinking water and also the spontaneous emergence of small scale entrepreneurs in the bottled water production [3], the need for this study becomes very important. This study therefore, focuses on determining the bacteriological and physicochemical qualities of bottled water, with the aim of characterizing and identifying the bacterial pathogens present in the bottled water; determining the prevalence of these pathogens and detecting physicochemical abnormalities in the bottled water and suggest possible measures to improve the quality of bottled water sold in Owerri, Nigeria [12].

In a society where there is an increasing demand for potable drinking water, also characterized by a spontaneous emergence of small scale entrepreneurs in the bottled water production [3] and with the depreciation in standard of production in a bid to maximize profit, the quality of available bottled water sold in Owerri is easily questionable. Though bottled water is readily available in Owerri, but there may be differences in their qualities due to different manufacturers and these raises a concern about the safety and potability of some of these products. Hence, there is need to study and evaluate the bacteriological and physicochemical status of bottled water sold in Owerri, so as to ascertain the possible risk of drinking bottled water sold in Owerri.

2. MATERIALS AND METHODS

2.1 Samples Collection

Twenty (20) bottled water sold in Owerri were selected randomly such that they represented the four major brands of bottled water (five bottled water for each brand) available or sold in Owerri. They were coded A to T for proper identification and transported to the microbiology Laboratory of the Department of Medical Laboratory Science, Imo State University, Owerri for processing and analysis.

2.2 Membrane filtration Technique, bacterial isolation, and Identification of Bacterial Isolates

The sterile filtration unit was set according to the manufacturers instruction, a sterile membrane filter was placed over a porous disk (the grid side up) with the aid of a sterile blunt ended forceps, the water sample was mixed thoroughly by inverting the bottle several times and 100ml of water was aseptically poured into the funnel, the water sample was drawn through the filter membrane by applying suction. Then the membrane from the filtration unit was removed aseptically using sterile blunt ended forceps and placed on the culture medium (Nutrient agar) in the Petri dish (grid side uppermost). The procedure was repeated for MacConkey and SSA agar. The plates were left for one hour and then incubated at 37°C for 48 hours. The plates were examined for bacterial growth and the colonies were enumerated after incubation [13]. All colonies isolated were sub-cultured from the mixed culture to fresh sterile media, to obtain pure culture of the isolates [3]. The isolates were then identified using biochemical tests like Gram staining, motility test, oxidase test, citrate utilization test, indole test and urease test.

2.3 Determination of Physicochemical Parameters

Determination of pH

The pH meter was turned on for at least 30 minutes before the test and then calibrated to 9.2 using the buffer and by adjusting the calibration knob. Also Calibrate the pH meter to 4.0 using the buffer and by adjusting the calibration knob. Afterwards, the pH of the samples were read by inserting the sample into the meter [14].

Determination of Total Chloride

1ml of K_2CrO_4 indicator was added to 100ml of the water sample, and titrated against 0.02N AgNO₃ till brick red precipitates were formed.

Formula: mg Cl⁻/l =
$$B.R \times N \times 35.45 \times 1000$$

Vol of sample (ml)

Where; B.R= Burette reading (amount of titrant used); N= normality of $AgNO_3$ and 35.45 = Equivalent weight of Chloride [14].

Determination of Conductivity

This is done with the conductivity meter. The conductivity meter was plugged to the source of power. 25ml of the sample was collected in a flat bottom flacks, then 25ml of deionised water was collected in another flat bottom flacks. The conductivity of the deionized water was read as the control, then the conductivity probe was placed in the flacks containing the water sample and its conductivity read from the conductivity meter [14].

Determination of Turbidity

The spectrophotometer was powered on, and the wavelength for turbidity (860nm) was selected and the programme number (750) was keyed in. After which 25 ml of distilled/de-ionized water (blank) was poured into one of the 25 ml cuvette to the mark, and was inserted into the cell compartment or light shield. The zero button was pressed to zero the spectrophotometer, then the water sample was vigorously shaken and 25 ml poured into the second cuvette. The blank cuvette was then replaced with the sample cuvette in the light shield then the READ button was pressed and the value of turbidity of the water sample was then digitally displayed in NUT (Nephelometric Turbidity Units) as stated by the International Organization of Standard [15].

Determination of Color

The photometer was put on, and the wavelength for color (420 nm) was selected. 10 ml cuvette was filled with distilled water to the mark and used as blank to zero the photometer. Then the cuvette was then replaced with another 10 ml cuvette filled with the water sample. The READ button was pressed to display the reading in PCU (platinum Cobalt Unit) [14].

Determination of Nitrate

The pH of a 25ml sample was adjusted to 5.0 and 9.0, 75ml of NH4Cl EDTA solution was added and mixed well. Nitrite is used to reduce Nitrate in the presence of Cadmium (Cd). This method uses commercially available cadmium granules coated with 2% copper sulphate (CuSO₄) packed in a glass column. The Nitrate (NO₃) produced was determined by diazotizing it with 2.0 ml color reagent containing sulphanilamide coupled with N-(1-napthyl)ethylenediamine diphydrochloride (NEDO) to form highly colored azo dye. Then the color developed was measured colorimetrically at 410nm wavelength. After this, a standard graph was plotted to obtain the factor [14].

Formula; mg NO₃/I = $O.D \times Factor$ Vol. of sample (ml).

Where; O.D= Optical density.

Determination of Nitrite

The pH of a 25ml sample was adjusted to 5.0 - 9.0 and 75ml of NH₄Cl⁻ EDTA solution was added and mixed well, then a 2 ml of color reagent was added to it. Distilled water is used as Reagent blank to set the instrument.

The color was read immediately at 543nm using UV-Visible spectrophotometer indicating O.D. Then a standard graph was plotted to obtain the factor [14].

Formula; mg NO₂/l= = $O.D \times Factor$ Vol. of sample (ml)

Where; O.D= Optical density

Determination of Iron

10L of the samples was dispensed into a small beaker and the pH was measured. The pH (must be within the range 1-10), was adjusted with sodium hydroxide (6.0 M) solution. Then 25 mL was treated with 0.1 mL of HNO_3 (0.1%v/v). Then, 5.00 mL was pipette into a pre-prepared test tube containing the buffer ammonium thioglycolate and thioglycolic acid. (Note: this buffer stabilizes the pH to 7.0). The test tube was tightly capped and mixed well until the reagent and sample were completely combined and then left 3 minutes. If the iron was present we will observe the formation of a purple solution. Afterwards the sample was measured in the UV-

Vis spectrophotometer with absorbance at 565 nm. The dissolved iron concentration was calculated from the calibration curve [16].

Determination of Phosphate

To suitable aliquots of stock standard solution, 1 mL of ammonium molybdate and 0.4 mL of hydrazine sulphate were added and the solution was made up to 10 mL with double distilled water in a standard measuring flask. The standard measuring flasks were kept in a water bath for heating for 30 min. The temperature of the water bath was set to 60° C. While heating, a blue color develops due to the formation of ammonium phosphomolybdate complex. After heating for 30 min the solution was cooled and its absorbance was measured at wavelength 830 nm. An experimental blank solution was used for carrying out correction for the baseline [15].

2.4 Data Analysis

Mean and Standard deviation was used to calculate the Mean Heterotrophic and Mean Coliform counts, mean values from the various Physicochemical analysis carried out. Bar chats were designed with a computer Software Package for Social Sciences (SPSS), to determine the relationship between various samples and their Mean Heterotrophic and Mean Coliform counts.

3. RESULTS

Fig. 1 represents the mean and standard deviation values for Total heterotrophic and Total coliform counts of the bottled water samples analyzed in this study. Sample F, J and R had a mean and standard deviation of 0.00±0.00 for both total heterotrophic and total coliform counts, whereas sample G and N had 0.00±0.00 for total heterotrophic count only, and samples A, I and M had a mean and standard deviation of 0.00±0.00 for total coliform count only.

Other samples recorded a mean and standard deviation of bacterial counts ranging from 1.50 ± 0.70 to 12.00 ± 2.82 and 1.50 ± 0.70 to 7.50 ± 2.12 for total heterotrophic and total coliform counts respectively.

Finally, it can be observed from the Fig. 1 that Sample L has the highest Total Heterotrophic Count.

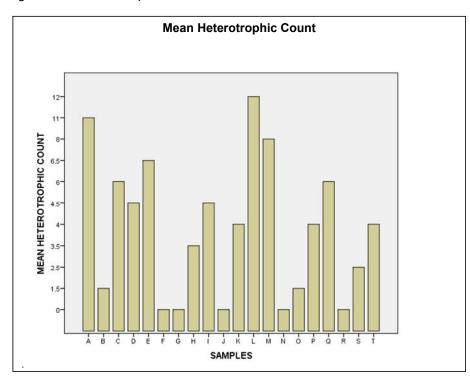


Fig. 1. Bar chart representing the mean heterotrophic count per 100ml of some selected brands of bottled

Mean Coliform Count

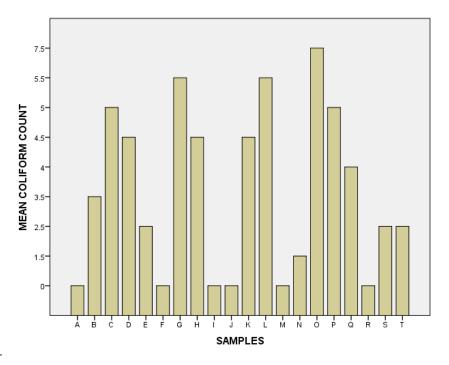


Fig. 2. Bar chart representing the mean coliform count per 100 ml of some selected brands of bottled water sold in Owerri

Fig. 2 represents the criteria for identification of the bacterial isolates from the bottled water samples analyzed in this study, describing their morphology, Grams staining and various biochemical characteristics. Four out of the five isolates are rod-shaped and the other is Cocci.

The first isolate is a Gram negative motile Rod shaped organism, which is Oxidase negative, Citrate negative, Urease negative, Indole positive thus confirming the isolate to be *Escherichia coli*.

The second isolate is a Gram negative motile Rod organism, which is Oxidase negative, Citrate negative, Urease positive, Indole positive thus confirming the isolate to be *Proteus specie*.

The third isolate is a Gram positive non-motile Cocci shaped organism, which is Oxidase negative, Citrate positive, Urease negative, Indole negative thus confirming the isolate to be *Staphylococcus*.

The fourth isolate is a Gram negative motile Rod shaped organism, which is Oxidase negative, Citrate negative, Urease negative, Indole negative thus confirming the isolate to be *Klebsiella spp.*

The fifth isolate is a Gram positive non-motile Rod shaped organism, which is spore forming, Oxidase positive, Citrate negative, Urease positive, Indole positive thus confirming the isolate to be *Bacillus spp.*

Table 1 represents the specific samples from which each of the isolates were found. And from the table it can be understood that *Escherichia coli* was isolated from most of the samples except sample A, F, I and N. *Proteus sp.* Was isolated from sample C, D, E, D, I, M, N, O, S and T. *Staphylococcus aureus* was isolated from sample I, K, N and R, then *Klebsiella spp* was isolated from sample B, C, E, G, J, M and Q and finally, *Bacillus spp* was isolated from sample A, D, G and N only.

Table 2 represents the Prevalence of the bacterial isolate among water samples. From the table it is observed that *Escherichia coli* had the highest prevalence (80%), having occurred in 16 different bottled water samples. Then *Proteus spp* had a prevalence of 50%, followed by

Staphylococcus aureus (20%), Klebsiella spp (35%) and Bacilllus spp (20%).

Table 3 represents the specific brand of bottled water from which the isolates were found. And from the table, it can be observed that *Escherichia coli* and *Proteus sp.* occurred in the four different brands of bottled water selected for this study. *Staphylococcus aureus* and *Klebsiella spp* occurred in all brands except in Z, and Bacillus *spp* occurred in all brands except in X. From the table also, it can be seen that W and Y have a 100% brand specific prevalence for the five isolate. And X and Z have a brand specific prevalence 80% and 60% respectively.

Table 4 represents the Mean and \pm Standard Deviation values of the Physicochemical analysis carried out in this study, and from the result it can be inferred that amongst all the brands of bottled water sold in Owerri, only W bottled was has a pH value within the reference range (NIS). The pH of the water samples ranged from 5.18 to 7.29 and only W conformed to the NIS. The turbidity of water samples also ranged from 0.53

- 1.64 NTU for all the selected bottled water samples. Conductivity measured at (µs/cm) also ranged from 16.00 - 262(µs/cm). Sample B, F and J (which represents X bottled water) has the lowest conductivity of 16µs/cm, while sample A, E and I (which represents W bottled water) has the highest conductivity of 262 us/cm. All the water samples had objectionable color, as none conformed to the Nigeria Industrial Standard (NIS). In the other hand, all the samples tested negative for phosphate, while Nitrate was not detected (zero) in most of the samples, with only samples B, F and J (which represents X bottled water) testing positive for Nitrate but at a very minute quantity. Same is applicable to Nitrite, to which almost all the samples tested negative except samples B, F and J (which represents X bottled water), which ranged from 0.34 - 0.35mg/L.

Finally, Iron was not detected (ND) in sample D and L. But in other samples, it ranged from 0.01 – 0.07 mg/L Fe. The Total Chloride ranged from 16.99 – 27.99 mg/L for the selected brands of bottle water.

lsolates No.	Grams stain reaction	Morphology (Cellular)	Oxidase test	Citrate test	Indole test	Motility test	Urease test	Probable Identity of Isolates
1	-	Rods	-	-	+	+	-	Escherichia coli
2	-	Rods	-	-	+	+	+	Proteus sp.
3	+	Cocci in clusters	-	+	-	-	-	Staphylococcus aureus
4	-	Rods	-	-	-	+	-	Klebsiella spp
5	+	Rods with spores	+	-	+	-	+	Bacilllus spp

KEY: + = Positive; - = Negative

Table 1. Sample specificity of identified bacter	ial isolates
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ISOLATES	SAMPLES																			
(Organisms)	Α	В	С	D	Ε	F	G	Н		J	Κ	L	Μ	Ν	0	Ρ	Q	R	S	Т
Escherichia coli	-	+	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+	+
Proteus sp.	-	-	+	+	+	-	-	+	+	-	-	-	+	+	+	-	-	-	+	+
Staphylococcus Aureus	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-	+	-	-

Isolates	Number of Occurrence	Prevalence
Escherichia coli	16	80%
Proteus spp	10	50%
Staphylococcus aureus	4	20%
Klebsiella spp	7	35%
Bacillus spp	4	20%

Isolates	W	Х	Y	Z	
Escherichia coli	+	+	+	+	
Proteus sp.	+	+	+	+	
Staphylococcus aureus	+	+	+	-	
Klebsiella spp	+	+	+	-	
Bacillus spp	+	-	+	+	
Brand Specific Prevalence	100%	80%	100%	60%	

Table 3. Specific brands of bottles from which isolates were found

KEY: + = *Positive (Present);* - = *Negative (Absent)*

 Table 5. Mean and ± standard deviation value of the physicochemical analysis of some selected brands of bottled water sold in Owerri

Parameters	NIS	W	Х	Y	Z
рН	6.50-8.50	7.28±0.00	5.94±0.00	5.18±0.00	5.92±0.00
Conductivity, µs/cm	1000.00	262.00±0.00	16.00±0.00	34.00±0.00	54.00±0.00
Turbidity, NTU	5.00	1.62±0.01	0.61±0.00	0.55±0.02	1.10±0.00
Color, PCU	5.00	18.00±0.00	0.00±0.00	2.00±0.00	17.00±0.00
Nitrate, mg/L	10.00	0.00±0.00	0.51±0.00	0.00±0.00	0.00±0.00
Iron, mg/L Fe	0.30	0.07±0.00	0.07±0.09	0.03±0.00	0.01±0.00
Total Chloride, mg/L	250.00	27.98±0.00	16.99±0.00	19.95±0.05	24.06±0.01
Phosphate (PO_4^{3-}), mg/L	5.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Nitrite, mg/L	1.00	0.00±0.00	0.34±0.00	0.00±0.00	0.00±0.00

Key: NIS= Nigeria Industrial Standard; NTU= Nephelometry Turbidity Unit; mg/L= Milligram per liter, PCU; Platinum-Cobalt Units.

4. DISCUSSION

The importance of good and safe drinking water cannot be overemphasized as regards the health of the population. The advent of bottled water has greatly reduced the occurrence of some water-borne/related diseases such as cholera, typhoid and dysentery. From this study, it was discovered that though W and Y had a brand specific prevalence of 100%, X and Z had a brand specific prevalence of 80% and 60% respectively. Furthermore, all the bottled water (100%) met the maximum limit of 10 coliforms per 100 ml of water allowed in potable water as stipulated by SON [17].

The total heterotrophic bacterial counts of the bottled water ranged from 0 to 12 cfu/ml while the total coliform counts ranged from 0 to 7 coliform/100ml. All the bottled water samples had heterotrophic bacterial counts less than 100 cfu/ml. Therefore, all the selected (20) bottled water samples assessed bacteriologically in this work, conformed to the World Health Organization standard for drinking water, this could be attributed to proper water treatment process.

However, only 6 (30%) out of the 20 selected bottled water had zero total coliform count

recommended for water to be used under emergency situation. The bacteria presence in the bottled water could have been derived from equipment used in production, improper capping of the bottled water, and post-production contamination during distribution and sales. This study sought to determine the quality of bottled water sold in Owerri. In contrast to other untreated drinking water sources, treated water is usually expected to contain no or only low levels of microorganism because of the antimicrobial effects of the disinfectant treatments applied, as reported by WHO [18].

The loads of heterotrophic bacteria, total coliform as well as the diversity of bacterial species obtained in this study were far lesser than those reported of sachet water and borehole water available in Owerri, as reported by Dike-Ndudim et al., [19] and Nwosu et al., [20]. This therefore indicates that bottled water is less contaminated when compared with sachet water and borehole water as reported by Sule et al., [21].

However, the presence of the isolates most of which are of Public Health importance, points to the possible risk of water borne diseases to which consumers are exposed. They can lead to great social-economic loss as stipulated by Nwosu et al. [20]. The *Staphylococcus* present in the bottled is known to be a commensal and it can be found on human skin surface, soil etc. therefore its presence in the bottled water samples could have resulted from postproduction contamination probably before or during capping, as stipulated by Sule et al. [3].

pH plays an important role in the survival of microorganisms in water. The pH values of bottled water samples selected for this study were not within the range of 6.5 to 8.5 allowed by SON for potable water, except Sample A, E and I (W bottled water). pH values which ranged from 5.18 to 5.94 were obtained in 3 other brands of bottled water sold in Owerri. This result can be compared to those reported by Sasikaran et al., [22] where pH values which ranged from 4.11 to 7.58 were obtained in 22 brands of bottled water sold in Jaffna Peninsula. It can also be contrasted with that reported by Sule et al., [3]. The consumption of acidic water can be injurious to the gastrointestinal tracts and therefore should not be taken.

The acceptable chloride content according to WHO is 250 mg/l as stipulated by WHO, [18]. The chloride content of all the samples, as well as their Iron content and turbidity was guite well below their limits, similar to those reported by Sule et al., [3]. The discoloration of some of the tested samples can be attributed to suspended solids containing minerals of iron that appear brownish in color. Even though the EPA says that the iron in the drinking water is safe to drink, the iron sediments, other trace impurities may support bacteria that are harmful, and these bacteria are mostly found in wells where the water has not been chlorinated or even under chlorinated processed water, as stated by Sreenivasareddy [16]. Furthermore, all of the selected sample failed the test for color and their pH were comparatively low, therefore are unsatisfactory.

5. CONCLUSION

From this study, it is discovered that there is a rapid deterioration in the quality of potable water with the increasing proliferation of bottled water companies in Owerri, Imo State. And from this, there is therefore a high risk of water borne diseases which is expected to rise sporadically without any intense effort and control. Furthermore, 20% of the samples used (which also represents 75% of the brands) for this study are acidic and therefore are not safe for consumption. It is therefore suggested that

stringent measures should be adhered to in the production of bottled water, the physicochemical parameters should be within the standard ranges, and information on the manufacturing date, expiry date and batch number should be written on the bottles for easy recall.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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