Microbial Quality Assessment of Commercial Bottled Water Brands in Major Markets in Awka, Nigeria

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Abstract The microbial quality of commercial bottled water purchased from Eke-Awka, Ifite-Awka, Amaudo-Awka, Permanent Site-Awka, Amaenyi-Awka, Agu-Awka and Okpuno-Awka Markets, Nigeria were assessed for total bacteria, total fungi, total coliforms and faecal coliforms. The temperature and PH values were also determined. The total bacteria ranged between 100 and 350 cfu/ml, total fungi between 10 and 50 cfu/ml, total coliforms from 0 to 30 cfu/100ml, faecal coliforms from 0 to 20 cfu/100ml, temperature from 27⁰C to 29⁰C, and PH from 5.7 to 6.3. The microbial isolates from the samples were *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Penicillium digitatum* and *Aspergillus niger*. Coliforms were not detected from the samples from Eke-Awka and Permanent Site-Awka Markets. The microorganisms were predominant in the samples from Okpuno-Awka Market (26%) while they had the least distribution in the samples from Eke-Awka Market (4%). The predominant bacteria were the gram-negative rods. *Escherichia coli* was the dominant bacterium (20%) while *Penicillium digitatum* was the dominant fungus (20%). Most of the samples did not comply with the standard set by regulatory bodies for drinking water and are therefore unsafe for drinking without adequate treatment.

Keywords Quality Assessment, Bottled Water, Major Markets, Nigeria

1. Introduction

Water can exist as liquid, gas and solid. It occupies about 70% of the earth's surface and is an essential commodity needed for the survival of humans, animals, plants and microorganisms. However, good and safe drinking water is unavailable to over one billion of the world population [1]. As important as water may be, its economic importance as a medium of transmission of diseases that afflict humans must

not be overlooked [2].

Almost two million people every year, the majority of whom are children die from water-related diseases [2] especially from diarrhea which remains the second leading cause of death among children under five years globally. It is estimated that almost one in five child's death is due to diarrhea, surpassing the death caused by AIDS, Malaria and Measles combined [2].

Microbial contamination is the most common reason for water to be deemed unsafe for drinking [1]. It is not possible to test drinking water samples for every water-borne pathogens and detection techniques are often expensive as well as time-consuming [3] and for this reason, indicator organisms are used to test for potential contamination by pathogens. The presence of *Escherichia coli* is used as an indicator for the presence of water-borne pathogens [1,4,5].

Ground water and surface water are the major sources of drinking water in both urban and rural areas. In Nigeria, ground water contamination is one of the least recognized environmental problems because ground water problems are not readily detected and pathways for contamination are not as noticeable as those affecting surface water [6]. Ground water is recovered from boreholes and deep wells [7] but it is however susceptible to microorganisms that impact human health directly.

Faecal contamination of borehole water occurs when faecal matter from a nearby septic tank infiltrates into the borehole chambers and introduces bacteria and other pathogenic microorganisms into such water drawn from the borehole. Microorganisms that are concerned with water-borne diseases include *Salmonella sp*, *Shigella sp*, *Escherichia coli*, *Vibrio cholerae* [8], *Entamoeba histolytica*, *Giardia lamblia*, *Balantidium coli*, *Cryptococcus parvum* and *Enteroviruses* of various clinical ailments which include *Poliovirus*, *Rotavirus*, *Hepatitis A virus* [9,10] and *Hepatitis E virus* [11].

Awka is the Capital City of Anambra State of Nigeria, with an estimated population of 301, 657 as of 2006 Nigerian census. It is a tropical zone which experiences both dry and wet seasons. The temperature in Awka is generally 27-30⁰C

between June and December but rises to 32-34°C between January and April. The city has a high demand for potable water though there is little or no government facility for the provision of such water, hence a significant percentage of the population depends on bottled water which sources are borehole waters for their daily water consumption, therefore the objective of this research was to assess the microbial quality of commercial bottled water brands in Major Markets in Awka, Nigeria.

2. Materials and Methods

2.1. Study Area

Awka Town in Anambra State, South-Eastern Nigeria is situated along the highway between Enugu and Onitsha. The Town's industries include printing, publishing, hotels, restaurants and Pharmaceutical firms. Its artisans are famous for wooden and metal crafts. The important commercial products of the area include yams, cassava, maize, palm products, peas, apples and oranges.

2.2. Sample Collection and Processing

Twenty one different brands of bottled water (3 from each market) were obtained from seven major markets in Awka. The major markets were Eke-Awka, Ifite-Awka, Amaudo-Awka, Permanent Site- Awka, Amaenyi-Awka, Agu-Awka and Okpuno-Awka. They were transported to the microbiology laboratory of Nnamdi Azikiwe University Awka on ice packs for analysis. The microbiological tests were carried out within twenty four hours after collection.

2.3. The Glass Bottles Sterilization and Media Preparation

The laboratory glass wares used were sterilized by heating in a hot-air oven at 160°C for one hour. The media which were prepared according to the manufacturer's instructions were sterilized by autoclaving at 121°C for fifteen minutes. The plates were prepared in duplicates.

2.4. Total Bacterial Count

The spread plate technique was employed. 0.1 millilitre of each water brand was spread with a sterile loop on the surface of a sterile nutrient agar (NA). The nutrient agar plates contained Ketoconazole at a concentration of 0.05mg/ml added to inhibit fungal growth. Incubation was carried out in an inverted position at 28°C for 24 hours after which the bacterial colonies that developed were counted and the result recorded. Each colony was purified by repeated subculturing on nutrient agar plates and stored in sterile nutrient agar slants for characterization and

identification.

2.5. Total Fungal Count

This was carried out using the spread plate method. 0.1ml of each water sample was dispensed into the centre of a sterile sabouraud dextrose agar (SDA) plate and spread evenly over the agar surface using a sterile glass rod. The agar plate had Chloramphenicol at a concentration of 0.05mg/ml to inhibit bacterial growth. Incubation was carried out in an inverted position at 28°C for 72 hours after which the fungal colonies that developed were counted and the result recorded. Each colony was subcultured on sterile SDA plates and later stored in sterile SDA slants for characterization and identification.

2.6. Total Coliform Count

The membrane filter technique was employed. 100ml of each sample were passed through a membrane filter and the filter aseptically transferred onto the surface of plates containing MacConkey agar (MA). Incubation was carried out in an inverted position at 28°C for 48 hours after which the coliform bacteria that developed were counted and the result recorded. Each colony was subcultured on sterile NA plates and later stored on sterile NA slants for characterization and identification.

2.7. Faecal Coliform Count

The membrane filter technique was also used. 100ml of each water sample was passed through a membrane filter which was thereafter transferred aseptically onto the surface of Eosin methylene blue (EMB) agar contained in plates. Incubation was carried out in an inverted position at 28°C for 48 hours after which the coliform bacteria that developed were counted and the result recorded. Each colony was subcultured on sterile NA plates and later stored on sterile NA slants for characterization and identification.

2.8. Faecal Streptococci Count

0.1ml of each water sample was introduced into test tubes of glucose azide broth and incubated at 45°C for 48 hours. The tubes were thereafter examined for acid production which was indicated by a change in the colour of the medium. Acid production is indicative of the presence of *faecal Streptococci*. The result was further confirmed by introducing 0.1ml of the samples from the tubes into sterile MacConkey agar and incubating at 45°C for 72 hours. *Faecal Streptococci* produces characteristic minute red colonies on this medium.

2.9. Clostridium Perfringens Count

0.1ml of each water sample was introduced into sterile

screw capped bottles containing differential reinforced Clostridial medium. The bottles were then incubated at 37°C for 48 hours. A positive reaction is indicated by the blackening of the medium. 0.1ml of the samples from the tubes was introduced into a tube of litmus milk medium and incubated at 37°C for 48 hours to confirm the presence of the organism. A positive reaction is indicated by the production of a stormy clot reaction.

2.10. Characterization and Identification of the Isolates

The colonial morphology and biochemical characteristics of the bacterial isolates were determined. Gram staining, Oxidase, Indole, Urease, Catalase, Methyl red, Voges proskauer, Coagulase, Citrate utilization, Sugar (glucose, sucrose, fructose and galactose) fermentation, Motility and Spore tests were carried out as described by Onuorah et al [12]. The isolates were identified following the scheme of Holt et al [13].

The fungal isolates were characterized and identified on the basis of their colonial and microscopic characteristics. The microscopic characteristics were determined using the lactophenol cotton blue staining and slide culture test, as described by Onuorah et al [14].

2.10.1. Slide Culture Test

A fragment of the aerial mycelia was picked with a sterile inoculating needle and inoculated on a slide containing prepared Sabouraud dextrose agar. The slide was thereafter incubated at 28°C for 24 hours after which it was stained with lactophenol cotton blue stain, covered with a coverslip and examined under the microscope.

2.10.2. Lactophenol Cotton Blue Staining

Two drops of lactophenol cotton blue dye were placed on a clean glass slide after which a fragment of the test fungal culture was teased out into the stain using a sterile straight wire. The slide was then covered with a coverslip, avoiding bubbles, and examined under the microscope. The isolates were identified following the scheme described by Oyeleke and Manga [15].

2.11. pH and Temperature Determination

The pH values of the samples were taken using a pH meter that was standardized with buffers 4 and 7. 100 ml of each sample were introduced into a beaker and the pH meter inserted into it. The pH values were then read and recorded.

The temperatures of the samples were taken with a thermometer. 100 ml of the sample were introduced into a beaker and the thermometer inserted into it. The temperature was thereafter read and recorded.

3. Results

The average temperature and pH values of the water

samples are shown in Table 1. The temperature ranged between 27°C and 29°C while the pH values were between 5.7 and 6.3.

Table 1. Average Temperature and pH values of the water samples

Sample Location	Temperature (°C)	pH
Eke-Awka	29	6.3
Ifite-Awka	28	5.7
Amaudo-Awka	29	6.2
Permanent Site-Awka	29	6.2
Amaenyi-Awka	28	5.7
Agu-Awka	28	6.2
Okpuno-Awka	27	6.3

The average microbial counts of the water samples are presented in Table 2. The total bacterial counts were between 100 cfu/ml and 350 cfu/ml, the total fungal counts between 10 cfu/ml and 50 cfu/ml, total coliform counts between 0 cfu/100 ml and 30 cfu/100 ml while the faecal coliform counts ranged between 0 cfu/100 ml and 20 cfu/100 ml.

Table 2. Average Microbial Counts of the Water Samples

Sample	Total Bacterial Counts (cfu/ml)	Total Fungal Counts (cfu/ml)	Total Coliform Count (cfu/100ml)	Faecal Coliform Counts (cfu/100ml)
Eke-Awka	100	10	0	0
Ifite-Awka	283	42	25	10
Amaudo-Awka	230	31	22	12
Permanent Site-Awka	115	15	0	0
Amaenyi-Awka	312	45	10	4
Agu-Awka	260	37	15	6
Okpuno-Awka	350	50	30	20

The microbial isolates in the water samples are shown in Table 3. They were *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Penicillium digitatum* and *Aspergillus niger*.

Table 3. Microbial Isolates in the Water Samples

Bacteria	Fungi
<i>Pseudomonas aeruginosa</i>	<i>Penicillium digitatum</i>
<i>Enterobacter aerogenes</i>	<i>Aspergillus niger</i>
<i>Escherichia coli</i>	
<i>Bacillus subtilis</i>	
<i>Klebsiella pneumoniae</i>	

The frequency of occurrence of the microbial isolates in the major markets is presented in Table 4. They were predominant in the samples from Okpuno-Awka Market (26%), while the distribution was lowest in the samples from Eke-Awka Market (4%).

Table 4. Frequency of occurrence of the Microbial Isolates in the Major Markets

Sample	<i>Pseudomonas aeruginosa</i> (n)	<i>Enterobacter aerogenes</i> (n)	<i>Escherichia coli</i> (n)	<i>Bacillus subtilis</i> (n)	<i>Klebsiella pneumoniae</i> (n)	<i>Penicillium digitatum</i> (n)	<i>Aspergillus niger</i> (n)	Total	% occurrence
Eke Awka	1	0	0	0	0	1	0	2	4
Ifite Awka	0	2	2	1	0	0	2	7	14
Amaudo Awka	0	1	1	1	2	2	0	7	14
Permanent Site Awka	0	0	0	2	0	1	3	6	12
Amaenyi Awka	0	1	2	0	1	3	0	7	14
Agu Awka	3	1	1	1	0	1	1	8	16
Okpuno Awka	4	2	4	0	0	2	1	13	26
Total	8 (16)	7 (14%)	10 (20%)	5 (10)	3 (6%)	10 (20%)	7(14%)	50 (100%)	100

n= number of Isolates

4. Discussion

The average temperature and pH values of the samples were between 27°C and 29°C and 5.7-6.3 respectively (Table 1). The average temperatures were within the mesophilic range and were suitable for the growth of heterotrophic organisms in the water samples. The temperature values conformed to the World Health Organization Standard for drinking water (25°C-40°C). The temperature of any water body affects the rate of proliferation of microorganisms [16]. The water samples were slightly acidic and hence supported the growth of microorganisms. The pH values were however below the WHO and Standard Organization of Nigeria guidelines for drinking water (6.5-8.5). Eniola et al [17] however, reported a pH range of 5.4-7.0 for borehole water samples stored indoor and outdoor in containers of different colours.

Bacteria and fungi were isolated from all the samples assessed (Table 2). *Coliforms* which are indicators of water quality were detected in all but the samples from Eke-Awka and Permanent Site Markets. Most of the water samples did not comply with the WHO [1] and SON [18] guidelines for drinking water since they contained the total *coliform* and *faecal coliform* bacteria in excess of the maximum permitted limits of 10 cfu/100 ml and 0 cfu/100 ml respectively. Their pH values were also higher than the recommended range of 6.5-8.5 [1,18].

The organisms isolated from the samples were *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Penicillium digitatum* and *Aspergillus niger* (Table 3). This result agreed with Birmingham et al [8], VanDerslice and Briscoe [19] and Wright et al [20] who reported the presence of coliforms in the water samples they studied. The dominant bacteria were the gram-negative rods while *Bacillus subtilis* was the only gram-positive bacterium isolated. Yeasts were not isolated from the samples. In addition, *Escherichia coli* was the dominant bacterium while *Penicillium digitatum* was the dominant fungus in the samples.

Microorganisms were predominant in the samples from Okpuno-Awka Market while the samples from Eke-Awka Market contained the lowest number of microorganisms (Table 4). This variation may be attributed to the packaging material, sanitary condition of the bottling environment, source of water supply and the hygiene status of the bottling staff.

Escherichia coli and *Penicillium digitatum* were predominant in the water samples while *Klebsiella pneumoniae* had the lowest frequency of occurrence (Table 4). *Escherichia coli* is commonly found in the lower intestines of warm-blooded organisms. Faecal-oral transmission is the major route through which pathogenic strains of this bacterium cause diseases. Though most *Escherichia coli* strains do not cause diseases, the virulence strain can cause gastroenteritis, urinary tract infections and neonatal meningitis. When *E. coli* is present in a water sample, other microorganisms can also be present.

Water that has *coliforms* or *Escherichia coli* is not safe for drinking [1, 18]. The presence of *faecal coliforms* in the water samples assessed is an indication of recent faecal pollution which suggests access to undesirable materials and could constitute a potential health hazard to the consumers. *Enterobacter aerogenes*, also a coliform bacterium is commonly found in polluted water, manure and soil. It's presence in some of the samples assessed may pose a public health risk.

Pseudomonas aeruginosa has been reported to be an opportunistic human and plant pathogens as well as a soil bacterium. Its ability to thrive under harsh conditions may have contributed to its being wide spread in nature. *Pseudomonas* infection is a particular problem in the environment as it is a nosocomial infection. *Klebsiella pneumoniae* is a human pathogen and can cause several diseases of man.

Although *Bacillus subtilis* is commonly found in soil, it is a normal gut commensal in humans. It can survive extreme temperature to which it is exposed as a result of its heat resistant spores. *Penicillium digitatum* and *Aspergillus niger*

are ubiquitous soil fungi present wherever organic material is available. They are also present in the air. *Aspergillus species* cause Aspergillosis with symptoms which include fever, chest pain and cough. *Faecal Streptococci* and *Clostridium perfringens* were not isolated from the samples indicating that the pollution of the water was of recent occurrence.

The result of this assessment showed that the microbial quality of most of the commercial bottled water sampled as well as their pH values were above the limit recommended by the World Health Organization and Standards Organization of Nigeria, therefore they must be adequately treated before bottling in hygienic environments. Filtration through sand bed filters, Chlorination and Ozonation are recommended.

5. Conclusions

The pollution of water bodies by microorganisms has become a major global problem because of the increase in water-borne diseases with the attendant high cost of water treatment. Since commercial bottled water is in high demand in Awka, there is urgent need to improve its quality. Proper treatment of the water samples before bottling, bottling in a hygienic environment as well as adequate post-bottling handling are recommended.

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