International Journal of Research and Review
www.ijrrjournal.com E-ISSN: 2349-9788; P-ISSN: 2454-2237

Original Research Article

Bacteriological Qualities of Drinking Water among Residents of Umuahia, Southeastern Nigeria

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ABSTRACT

Adequate supply of clean water is a necessity for sustaining human life and for achieving sustainable development. This study was carried out in Umuahia, Southeastern Nigeria to investigate the sources of water supply of the residents as well as laboratory analysis of the bacteriological qualities of water. A well-structured questionnaire was also used to interview the subjects in order to determine the sources of water supply and its utilization. The water samples were collected from streams, running tap water, underground well water, reservoirs from rain water, and reservoirs from borehole water. The water samples were transported in sterile containers to the laboratory for bacteriological analysis. Microbial isolates were characterized based on cultural (colonial), microscopic and biochemical methods. Results showed that the major sources of drinking water in the communities include borehole (36.1%), open well (2.9%), bottled water (4.3%) and stream (10.4%). The mean total bacteria count for Nutrient Agar (NA) was 7.6 x 10^6cfu/ml; Eosin Methylene Blue Agar (EMBA), 7.8 x 10^4cfu/ml; Salmonella-Shigella Agar (SSA), 3.4 x 10^3cfu/ml; Blood Agar (BA), 5.3 x 10^5cfu/ml; MacConkey Agar (MCA), 1.6 x 10^7cfu/ml. Out of 230 samples collected, *Enterococcus faecalis* was isolated on 15% of the samples followed by *Klebsiella pneumonia* (9%). Others include *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Micrococcus roseus* at 8% each. The least bacterial isolates were the *Streptococcus pyogenes*, *Escherichia coli* and *Aeromonas sp.* at3% each. In conclusion, borehole water was the major source of water among the households in Umuahia and the major bacterial isolates in the water samples were *Enterococcus faecalis*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Micrococcus roseus*. It was recommended that people should treat (boil) their water properly before drinking in order to ensure total destruction of bacteria organisms.

Keywords: Water, Bacteria, Agar, Organism, Fecal

INTRODUCTION

Water is one of the most important substances on earth. All plants and animals must have water to survive. If there was no water, there would be no life on earth. It is important that the water which people drink and use for other purposes is wholesome. This means that the water must be potable water (free from disease-causing organisms and chemicals). Disease-causing organisms and chemicals can find their way into water supplies and when this happens the water becomes contaminated thus leading to various diseases to those that come in contact with it. The benefits of having access to an improved drinking water source can only be fully realized when there is also access to improved sanitation and adherence to good hygiene practices. [1] Water supply means the provision by public utilities, commercial organizations, community endeavors or by individuals of water, usually by a system of pumps and pipes. A recent study [2] by the United Nation Children's Fund (UNICEF) says only two out of every ten schools in Nigeria have basic water supply and functioning sanitary services. Water being a key natural resource is therefore needed in good quality and quantity. An adequate supply of wholesome water is a prerequisite for sustaining human life, maintaining ecological systems and for achieving sustainable development. [3] For a large percentage of the world's population, drinking water supplies and sanitation services are neither safe nor adequate. Currently, over 1000 million people do not have access to an adequate supply of safe water for household consumption and nearly 3000 million lack a sanitary means of excreta disposal. [4] The provision of safe water and the management of wastewater have had a central role in reducing the incidence of many waterborne enteric infections or water-related communicable diseases.

One of the major achievements of the past 150 years is the extent to which diseases associated with water have become of minor significance in the mortality and morbidity statistics of most developed countries and of some developing countries (especially for richer groups living in major cities). [5] The diseases associated with contaminated water, however, remain serious public health problems for most of the world's population. At the same time, water shortages in many countries are now imposing serious constraints on municipal and community development, as well as on the expansion of food production and the growth of industry. Countries with relatively low per capita levels of available freshwater are finding it difficult to meet the increasing demands for fresh water from expanding populations and the growing demands from agriculture and industry. [6] Surface water supply is derived from waters that are found on earth's surface. They include ponds, brook, stream, rivers, sea and ocean. [7] In Nigeria, most communities depend on surface water as it is their major source of water supply.

Bacteriological analysis of water is a method of analyzing water to estimate the number of bacteria present and if need be, to determine by characterizing the type of bacteria present. [8] This kind of water analysis represents one aspect of water quality. It is a microbiological analytical procedure which uses samples of water and from these samples determines the concentration of bacteria. It is then possible to draw inferences about the suitability of the water for use from these concentrations. This process is used, for example, to routinely confirm that water is safe for human consumption or that bathing and recreational waters are safe to use. Microbial contamination is by far the most serious public health risk associated with drinking-water supplies. It is impractical to analyze water for every individual pathogen, some of which can cause disease at very low doses. Instead, since most diarrhoea-causing pathogens are faecal in origin, it is practically important to analyze water for indicator species that are also present in faecal matter. The use of indicator organisms in the bacteriological analysis of water has remained the mainstay of water bacteriology. The most reliable methods are direct plate count method and membrane filtration method. [9] Endo Agar/broth is used for the estimation of coliform bacteria in water samples using the membrane filtration technique, while violet red bile agar (VRBA) is used in the direct plate count method. These media contain lactose which is usually fermented by lactose fermenting bacteria producing colonies that can be identified and characterized. Lactose fermenting organisms produce colored

colonies while non-lactose fermenting organisms produce colorless ones. Because the analysis is always based on a very small sample taken from a very large volume of water, all methods rely on statistical principles. [10]
For many years, total coliforms have been used as indicators in evaluating water quality for several water uses with respect to faecal contamination. [11] Not all coliforms are from faecal source. Hence, feacal coliforms and pathogenic forms such as *Escherichia coli* are now used largely as bacteriological indicators. [12] The term “total coliforms” refers to a large group of Gram negative, rod-shaped bacteria that share several characteristics. The group includes thermo-tolerant (ferment lactose and produce gas at 45.5°C) coliforms and bacteria of faecal origin as well as some bacteria that may be isolated from environmental sources. Thus the presence of total coliforms may or may not indicate faecal contamination. [13] In extreme cases, a high count for the total coliform group may be associated with a low or even zero count for thermo-tolerant coliforms. Such a result would not necessarily indicate the presence of faecal contamination. It might be caused by entry of soil or organic matter into the water or by conditions suitable for the growth of other types of coliform. In the laboratory, total coliforms are grown in or on a medium containing lactose at a temperature range of 35-37°C. They are provisionally identified by the production of acid and gas from the fermentation of lactose. [13] Unlike coliforms from environmental sources, coliforms that come from faecal matter can tolerate higher temperatures. These are more closely associated with faecal contamination than total coliforms. The most specific indicator of faecal contamination is *E. coli*, which unlike some faecal coliforms never multiplies in the aquatic environment. [14] *E. coli* is now internationally acknowledged as the most appropriate indicator of faecal contaminant. In source water, its level of occurrence is correlated with the inputs of faecal contamination. [15] Other organisms used as indicators of faecal contaminants of water include *Streptococci, Enterococci, Clostridium perfringens, Pseudomonas aeruginosa*, Hydrogen sulphide (H_2S) - producing bacteria, coliphages and other bacteriophages. [14, 16] This study was carried out to investigate the sources of water supply and the bacteriological qualities of the water samples in Umuahia, Southeastern Nigeria.

MATERIALS AND METHODS

Collection of samples

The study was a laboratory study carried out to investigate the bacteriological qualities of water in Umuahia, Southeastern, Nigeria. A well-structured questionnaire was also used to interview the subjects in order to determine the sources of water supply and its utilization by residents of Umuahia. Water samples were collected from three communities in Umuahia Local Government Area, Southeastern Nigeria. The sampling points included streams, running tap water, underground well water, reservoirs from rain water, and reservoirs from borehole water. The water samples were transported in sterile containers to the laboratory for analysis.

Preparation of Media and Diluents

Nutrient Agar (NA), Eosin Methylene Blue Agar (EMBA), Salmonella Shigella Agar (SSA), Blood Agar (BA) and MacConkey Agar (MCA) were prepared according to manufacturer’s specification. [17] Nutrient agar was used in the isolation of heterotrophic bacteria. MacConkey agar selects for coliforms (*Escherichia coli*) and faecal coliforms. EMBA is highly selective for *Escherichia coli* and other Enterobacteriaceae, whereas SSA and Blood Agar were used for the cultivation of *Salmonella and Shigella* and haemolytic bacteria respectively. Physiological saline used as diluent was prepared by dissolving 9.8g of sodium chloride in 1000 ml of distilled water and dispensed in 9 ml portions. Both diluents and media were sterilized in an autoclave at 121^0C for 15 minutes. One milliliter (1 ml) of water sample was serially diluted in 9 ml of sterile physiological saline and swirled to mix thoroughly. An aliquot (0.1ml) of appropriate dilution was inoculated into the pre-sterilized and surface dried medium. Inocula were spread evenly to ensure uniform and countable colonies. Plates were incubated at ambient temperature for 24-48 hours.

Characterization and identification of microbial isolates

Microbial isolates were characterized based on cultural (colonial), microscopic and biochemical methods with reference to standard manuals. [18] The identities of the isolates were cross-matched with reference to standard manuals for the identification of bacteria. Biochemical tests carried out include catalase test, coagulase test, oxidase test, sugar fermentation, hydrogen sulphide production test,urease test, IMViC test, indole test, and citrate utilization test.

RESULTS

A total of 230 subjects and water samples were used for this study. Table 1 showed that the major sources of drinking water in the communities include borehole (36.1%),open well (2.9%), bottled water (4.3%) and stream (10.4%). Table 2 represents the result for the mean bacteria Counts (CFU/ml/g) of water samples. The mean total bacteria count for Nutrient Agar (NA) was 7.6 x 10^6; Eosin Methylene Blue Agar (EMBA), 7.8 x 10^4; Salmonella-Shigella Agar (SSA), 3.4 x 10^3; Blood Agar (BA), 5.3 x 10^5; MacConkey Agar (MCA), 1.6 x 10^7. The bacteria isolates found in the water samples are *Enterococcus faecalis* (15%), followed by *Klebsiella pneumonia* at 9%. Others include *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Micrococcus roseus* at 8% each. The least bacterial isolates were the *Streptococcus pyogenes*, *Escherichia coli* and *Aeromonas sp.* at3% each (Figure 1).

Table 1: Distribution of the sources of drinking water

Source of drinking water	N	%
Piped water	6	2.6
Borehole	83	36.1
Covered well	4	1.7
Open well	7	2.9
Spring	9	3.7
Rainwater catchment	7	2.8
Lake/ stream	24	10.4
Bottled water	10	4.3

Table 2: Mean and standard deviation of Total Bacteria Counts (CFU/ml) of water Samples

Sample	Total counts on NA	Total counts on EMBA	Total counts on SSA	Total counts on BA	Total counts on MCA
Mean	7.6 x 10^6	7.8 x 10^4	3.4 x 10^3	5.3 x 10^5	1.6 x 10^7
Standard deviation	1.6 x 10^7	2.4 x 10^5	1.8 x 10^4	1.9 x 10^6	8.5 x 10^7

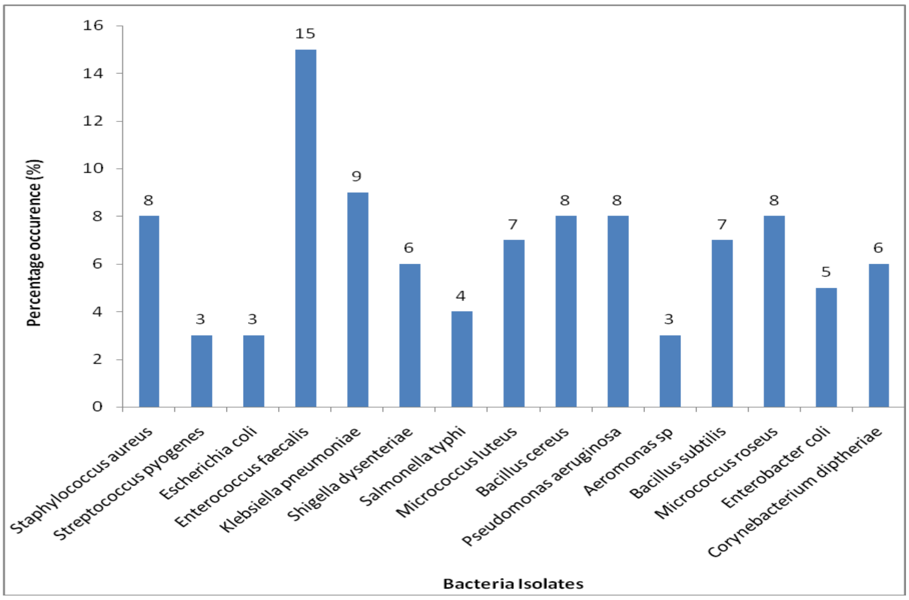

Figure 1: Distribution of bacterial isolates in water samples

DISCUSSION

Borehole water was the main source of water for the majority of the subjects in this study. This is a common source of water in most urban centers in Nigeria. Being a state capital, many houses in Umuahia have boreholes as the source of water for the inhabitants. The borehole water is not free from contamination with bacteria. Underground water can get contaminated from the soil or from runoff. Figure 1 showed the bacterial isolates from the water samples. *Enterococcus faecalis* was the most prevalent bacteria isolated. These bacteria are very resilient, so they can survive in hot, salty, or acidic environments. *E. faecalis* bacteria don't usually cause problems in healthy people. But people with underlying health conditions or a weakened immune system are more likely to get sick. *E. faecalis* infections spread from person to person through poor hygiene. Because these bacteria are found in feces, people can transmit the infection if they don't wash their hands after using the toilet. The bacteria can get into the underground water. [19] Symptoms of *E. faecalis* infection include fever, chills, fatigue, headache, abdominal pains, nausea and vomiting. [20]

Staphylococcus aureus was isolated in 8% of the water samples. It is a commensal and opportunistic pathogen that can cause wide spectrum of infections, from superficial skin infections to severe, and potentially fatal, invasive disease. [21] They can survive in water bodies in room temperature. The bacterium is found in water and is of major concern in public health programs worldwide. [22] A typical Water-Borne Disease caused by *S. aureus* has a rapid onset following ingestion of contaminated water. This is due to the production of one or more toxins by the bacteria during growth at permissive temperatures. [23] However, the incubation period of Staphylococcal Water-Borne Disease depends on amount of toxin ingested. The onset is abrupt. Symptoms include hyper salivation, nausea, vomiting, and abdominal cramping with or without diarrhea. If significant fluid is lost, physical examination may reveal signs of dehydration and hypotension. [24]

Klebsiella pneumonia was isolated in 9% of the water samples. They have been found in a variety of environmental situations, such as soil, vegetation, or water, and they influence many biochemical and geochemical processes. They have been recovered from aquatic environments receiving industrial wastewaters, plant products, fresh vegetables, food with a high content of sugars and acids, frozen orange juice concentrate, sugarcane wastes, living trees, and plants and plant byproducts. [25] *Klebsiella* has been isolated from the root surfaces of various plants. *Enterobacter coli*, a member of *Enterobacteriaceae*, were isolated in 5% of the water samples. Before the widespread use of antibiotics, *Enterobacter* species were rarely found as pathogens, but these organisms are now increasingly encountered, causing nosocomial infections such as urinary tract infections and bacteremia. In addition, they occasionally cause community-acquired infections. [26]

Shigella dysenteriae are Gram-negative, non-spore forming, non-motile, straight rod-like members of the family *Enterobacteriaceae*. The bacterium was isolated in 6% of the water samples. The incubation period is 1 to 4 days. The disease usually begins with fever, anorexia, fatigue and malaise. [27] They can be found in water bodies and cause the disease Shigellosis. Patients display frequent bloody stools of small volume and abdominal cramps. Twelve to 36 hours later, diarrhea progresses to dysentery, blood, mucus and pus appearing in feces that decreases in volume. [28] Although the molecular basis of shigellosis is complex, the initial step in pathogenesis is penetration of the colonic mucosa. The resulting focus of *Shigella* infection is characterized by degeneration of the epithelium and by an acute inflammatory colitis in the lamina propria. Ultimately, desquamation and ulceration of the mucosa cause leakage of blood, inflammatory elements, and mucus into the intestinal lumen. Under these conditions the absorption of water by the colon is inhibited and the volume of stool is dependent upon the ileocecal flow. As a result, the patient will pass frequent, scanty, dysenteric stools. This has been reported in related studies. [29]

In conclusion, borehole water was the major source of water among the households in Umuahia. The major bacterial isolates in the water samples were *Enterococcus faecalis*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Micrococcus roseus*. It is recommended that people sterilize their water properly before drinking in order to ensure total destruction of bacteria organisms.

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How to cite this article: Okereke EE, Amadi, COA, Iro OK. et.al. Bacteriological qualities of drinking water among residents of Umuahia, Southeastern Nigeria. International Journal of Research and Review. 2019; 6(12):324-330.

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