



Bacteriological assessment of Satchet water from different sources in Umuahia metropolis

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Abstract

20 satchets water samples were randomly collected. The water samples were analysed by the Multiple Tube Method, coliform counts were determined by the Most Probable Number (MPN) technique; and result ranged from 0 - >18 orgs/100 ml. Isolates were further identified by routine laboratory techniques. *Escherichia coli* was predominant in 4(20%) SW. Other isolates include *Salmonella* and *Enterobacter* spp, 4% each in the total samples, followed by *Klebsiella* spp, 3%; *Proteus* spp and *Bacillus* spp with 2% each in 20 samples. The result indicates a recent and (perhaps continuous) faecal pollution arising from poor hygiene and failure of treatment processes. This does not meet the WHO standard for potability.

Keywords: Bacteriological assessment , Satchet water, Umuahia Metropolis.

Introduction

Water is essential to the life of all living organisms including microorganisms. It plays an important role in the structure and function of the human body and remains the medium for biological and chemical processes in all living things. It constitutes about 70% of the body weight of every healthy adult (Sotade, 2003; Legislation, 1997). From the United Nations records on the World water situation, while 70% of the earth is covered by water, the earth's entire water resources are estimated at 1.5 billion km³ of which 97.5% of this is salt water that cannot be consumed directly. Of the remaining 2.5%, almost three-quarters is trapped in glaciers and ice sheets; fresh water accounts for 0.3% of the total water resources. Pure water of 100% purity could be very hard to find in any natural sources of water. It should not contain concentration of microorganisms, parasites or any other substances, which constitutes a potential health

risk (Council Directive 98/83/EC, 1998). However, in practical terms drinking water will always be "contaminated" with a small number of harmless bacteria and numerous chemicals, some of which may even be regarded as beneficial to either its taste or its health giving properties. But indeed, water of very good quality should be tasteless, odorless and colourless, with pH of about 7.0. And average man's intake is about 2.6 litres.

The National Agency for food and drug Administration and Control (NAFDAC) regulations agree with the World Health Organization (WHO) standards for portable water.

Illnesses ensue from consumption of water contaminated by pathogenic bacteria, viruses, parasites, algae and protozoa, including other aquatic

micro-lives. Such illnesses may include diarrhoea, salmonellosis (typhoid and paratyphoid fevers), cholera and dysentery. These may present symptoms like nausea, stomach cramps, vomiting, low grade fevers which may begin from two to ten days after drinking the contaminated water. Some pathogenic viruses can also be water-borne, notably those of poliomyelitis, hepatitis A and rotavirus. Parasitic diseases include schistosomiasis, guineaworm infections (dracontiasis) and toxoplasmosis. Among the protozoan diseases frequently encountered are amoebic dysentery, giardiasis and balantidial dysentery. People with toxoplasmosis may have fever, swollen glands and loss or blurring of vision.

Unsafe water consumption brings about high infant and child mortality; and for those who survive into adulthood: poor health, loss of productivity and shortened life are amongst the problems likely to arise. Persons with suppressed immune systems (such as HIV/AIDS patients, those who have had organ or bone-marrow transplant or who have had cancer treatment) are at risk from water-borne diseases. For such people, infection may be more, and may become life-threatening. Infants, the very elderly and those whose health is fragile due to chronic diseases are also more vulnerable to more serious complications (Bichi et al., 2002; BC-Health Files, 2000).

Drinking water that does not comply with WHO standards may have become contaminated by human or animal faeces, for example, raw sewage entering a water supply during times of flooding has enormous potentials for spreading microbial diseases (Cheesbrough, 2000; Bichi et al., 2002).

Epidemiological investigations of wastewater-contaminated drinking water supplies near irrigation sites have provided evidence of infectious disease transmission, according to EPA (1992) report.

Infection of water containing certain organic compounds may result in acute or chronic health effects. The organic make-up of raw water includes naturally occurring humic substances, faecal matter, kitchen wastes, liquid detergents, oils, grease, and other substances that in one way or another become part of the sewage stream that may enter into water. Industrial and residential wastes can contribute significant quantities of synthetic organic compounds. The spreading of slurry and manure has also resulted in infections in human (Watkins and Cameron, 1991). Our area of investigation may be prone to these sources of contamination due to its rate of

urbanization. Underground wastewater in communities are polluted by materials from human and industrial activities; underground leakages (of refineries in some areas); untreated effluents from industries (Anaebonam, 1995).

Sotade (2003) reported that about 90% of sewage and 70% of industrial wastes are discharged, untreated, often polluting the usable water supply. Consequently, at any time, people suffering from water-borne disease occupy more than half of the world's hospitals. According to EU Directives (1997) in urban areas one of the greatest threats to groundwater is that posed by the noxious leachate generated by domestic refuse in dumps or landfills. Other sources of urban threats include accidental spillages, leaking sewages and underground storage containers. In rural areas badly constructed or sited septic tanks and silage effluent constitute the principal pollutants. The spreading of slurry has been shown to cause contamination of drinking water supply resulting in a small outbreak of *Cryptosporidium* in Scotland in 1988 (Watkins and Cameron, 1991). It is noteworthy that waste from human beings living near ponds and rivers has led to the pollution of surface water bodies. Although water may appear sparkling clean and clear, it may harbour harmful germs such as intestinal parasites, liver flukes, hookworms, amoeba and lots more. Even, some wells and boreholes are dug within the proximity of sewage systems and septic tanks, causing surface contamination from these sources of water (Sotade, 2003). Left to search for drinkable water themselves, many people stand a very high risk of contracting water-borne diseases. This is also true for unscrupulous industrialists (manufacturers) and water vendors who sell substandard bottled and packaged water.

Surface water provides a valuable resource for drinking water as well as recreation. Sudden deterioration following pollution can render an otherwise adequate treatment inadequate. Reported farm pollution incidents have increased steadily, and the highest of the incidents (55%) were related to cattle slurries in 1988 according to (Watkins and Cameron, 1991). Problems arise from the containment of as much as two million liters of slurry during a wet winter.

Contamination can enter water bodies in a variety of ways. These include discharges from sewage works and industrial plant at identifiable point sources; intermittent discharges from sources such as storm flows and land run off; continuous leaching from

surrounding ground including pollutants in the groundwater entering the system; deposition from the air, eg, accidental or deliberate spillage or dumping and release from dead or decaying, aquatic flora and fauna (EU Directives, 1997). Most cases of water pollution in Ireland were found to be caused by discharges into waters, non-toxic organic matter, sewage, manure slurry, agriculture/food production wastes and silage effluent, which lead to deoxygenation. Eutrophication (over-enrichment of waters by nutrients— mainly nitrates and phosphates) derived from sewage effluent or through the decaying of these nutrients from manure slurries and artificial fertilizers, can lead to the stimulation of weed and algal growth which can ultimately cause deoxygenation. There has been a gradual increase in Eutrophication of lakes and rivers, caused mainly by inadequate sewage treatment and agricultural intensification.

One of the problems and concerns of developing countries is faecal-oral diseases related to water supplies and sanitation of communities (WHO, 1997). Nigeria has not been able to overcome this problem of inability to provide adequate good quality drinking water to her cities. In Umuahia Metropolis, — the capital city of Abia State (in the South East of Nigeria), is under intense urbanization which can naturally lead to slum formation. WHO (1997) declares that: "all people, not withstanding their stage of development and social and economic condition, have the right to have access to drinking water in quantities and of a quality equal to their basic needs".

Obviously irked by substandard treatment of such water, the Federal government of Nigeria banned the sale of water packaged in polythene sachets (popularly known as pure water) not registered with National Agency for Food and Drug Administration and Control (NAFDAC). This has however, not deterred the labyrinth of producers who make much profit from quick turnover of the "commodity". Some concerned consumers have revealed that even those approved by NAFDAC do not always keep to required standard. Once they have paid for registration and received certificates of approval from NAFDAC they often resort to packaging any type of water when they are no longer under supervision.

The environment where the water is packaged is also of equal concern. Only a few of the major producers have complete in-house facilities for producing the water containers. Polythene and other packaging materials/bottles are approved internationally for water

and foodstuff. Many brands of bottled water are adulterated daily by unscrupulous packagers.

WHO (1995) has stated that several millions of people living in Africa do not have access to safe water due to poor hygienic toilet facilities, simply, because the governments in these parts of the world lack the required commitments. For this reason, water related illnesses have continued to thrive in the region. Changes, such as intensive agricultural practices, industrial growth and increasing urbanization are all contributing to a major increase in the pollution load reaching our waterways. Water is indispensable in the society because, without a reasonable quantity of it in our body, we dehydrate. The body's need for water physiologically supersedes its need for food. Needless to emphasize that effective transformation of our economy is never possible when the most basic necessity of life like water is neglected.

The chronic shortage of water is the same in both rural and urban areas. It is more worrisome, however, in the rural areas where people trek many kilometers in search of water for domestic use. This is because the hawkers or water delivery trucks are almost absent; coupled with the fact that the rural dwellers do not have enough money to buy water. In many areas, the only source of water is the flood after rains .

In the urban areas, people spend a substantial part of their income on buying water; as water selling has become a booming business. A barrel of water sells for about ₦300 (three hundred naira) in the rainy season and ₦500 (five hundred naira) in the dry season. This is economically exacting on civil servants whose incomes are lean. Against this backdrop of scarcity of potable water, it is not surprising therefore, that more than two million Nigerians contract guinea worm diseases annually (Nwokoro, 1994). Over one million are temporarily incapacitate while many more may suffer permanent disability from diseases of bad water they drink.

Provision of clean water to Nigerians has always been one of the greatest challenges of Nigerian government. Successive governments have applied various methods to solving this problem, but have been hindered mostly by finance, lack of commitment and political will. Statistics show that till 1999, less than thirty per cent (30%) of Nigeria's rural and urban population had access to safe drinking water. Even those that have did not get the water regularly (Olugbayo, 2003).

The implication of this is that people are exposed to a lot of hardship as well as diseases like diarrhoea, dysentery, typhoid fever and other water-related diseases, some of which are life-threatening; which exert economic burden on the people. International Health Agencies attribute eighty per cent (80%) of various diseases in our hospitals to water-borne vectors (Nanan et al., 2003). Any programme aimed at improving water supply and sanitation facilities, providing appropriate hygiene education will likely give significant health benefits. Improvement of public health is the strongest and most frequent argument put forward in support of water and sanitation projects. Inadequate water and sanitation adversely affect the health and socio-economic development of a people. Water quality has been recognized as one of the foundations for any health improvement strategy (Nanan et al., 2003).

Global water consumption has increased tenfold in the last century. Over a billion people in the world have no access to safe drinking water; 2.4 billion lack adequate sanitation (WHO, 2000; Avery, 2003). The result is that 3 million people die from preventable water-borne diseases every year.

Public water is of concern just as private water supply. Recent developments in the private water business, particularly packaged water give cause for concern. The water situation in Umuahia is a typical example of a Nigerian city, with constantly dry public water taps or none at all; and residents are forced to source for drinking water anywhere. Some people have dug private boreholes as major sources of water for domestic use, while many depend on water from rivers, streams and harvested rain water. The safety of these sources of water is questionable. This is because, water is so easily contaminated and it soon becomes unfit for human consumption (Sotade, 2003).

Water packaged in polythene sachets, popularly called "Pure water" is gaining acceptance in Umuahia as with other parts of Nigeria. Travelers seem to patronize the hawkers more. But the unhygienic preparation of some of these could lead to epidemics and ill health. It is part of the efforts to correct this that the government of Nigeria enacted a decree empowering the National Agency For Food and Drug Administration and Control (NAFDAC) to regulate the business by granting licenses to eligible producers; renewable every four years. An operator without the license could be prosecuted for selling any type of packaged water.

Water shortage in major Nigerian cities has become such a lingering problem that has attracted the attention of the International Community, including non-governmental organizations (NGOs). Some countries and governments have demonstrated concerns by donating huge equipment and grants for water drilling and the sinking of boreholes in the worst-hit communities, e.g. the multi-billion naira ADIYAN water project in Oke-Aro.

Anaebonam, (1995) reveals that various levels of governments have capitalized on these donations/grants to propose gigantic water projects that were later abandoned due to carelessness by the managers or inadequate funding. These failures often not only dashed people's hopes but sometimes led to apathy among the donors. The Lagos State government took advantage of these grants from World Bank to embark on the multi-billion naira ADIYAN Water project in Oke-Aro. As gigantic as this scheme was, it never got completed on schedule. The Adiyam project which started in 1988, could not go beyond 60% after about 10 years.

The UNICEF has, however been making laudable efforts towards the provision of potable water for the rural dwellers, but this is hardly adequate. The communities have been making meaningful contributions towards water development what has been achieved so far is not really remarkable because it is still inadequate.

It is impracticable to attempt directly to detect the presence of all the different kinds of water-borne pathogens, any of which may be present only intermittently. Therefore, it is more usual to monitor for the presence of indicator organisms. These indicators are usually common intestinal commensal bacteria which are universally present in, and excreted in large numbers by, man and animals though found in other sources in small numbers. These are called coliforms, more appropriately referred to as "Typical" or "Faecal" (eg, *Escherichia coli*) or "Atypical" (eg, *Klebsiella aerogenes*, *Citrobacter* spp and *Enterobacter* spp), which may also grow in the soil and on vegetation; and therefore may often be present in water that are subject to excretal pollution (Mackie and McCartney, 1996). In themselves, they are not dangerous (to a healthy individual), but their presence indicates that faecal matter may have entered the water supply. Such supply source is therefore liable to contamination with dangerous intestinal pathogens. *Streptococcus faecalis* and *Clostridium perfringens* have similar significance, though the latter

(Clostridium), being a sporing organism survives longer than other faecal organisms. Therefore their presences suggest that a contamination is not a recent one (but has a remote cause). It is noteworthy that the "Typical" or faecal coliform (*E. coli*) dies in water in a few days or weeks after leaving the animal intestine (Cheesbrough, 2000; Mackie and McCartney, 1996).

It is true that the presence of coliforms is a strong evidence of faecal pollution, however, their absence does not exclude such impurity. There are a few accounts when it is not safe to assume that water supply has not been contaminated: where Pit latrines, Soakaways, including Sewage and Refuse dumps (Heaps) are situated very closely to the water source.

An important point to note therefore is that it is not the total bacterial count (TBC), however, which is of primary importance in assessing the suitability of a water supply for human consumption; but the possibility that it contains organisms capable of causing diseases in those who drink the water. This does not rule out (undermine) the World Health (WHO) standard that Heterotrophic Colony Counts (HCC) are useful indicators that should be used in conjunction with Total Coliforms and the presence of *E. coli* as routine tests to monitor the suitability of drinking water (Ratto et al., 1989). By implication, therefore, the test for index of the pollution must be carried out on a quantitative basis. The more reliable indicator of faecal contamination, *E. coli*, comprises - approximately 90% of the total coliforms found in faeces.

This investigation intends to achieve its objectives by using the Multiple Tube Method, also called the Most Probable Number (MPN). It is based on an indirect assessment of microbial density in the water sample by reference to statistical tables to determine the MPN of microorganisms present in the original sample. Also, this technique is used extensively for drinking water analysis (both routinely and for research investigation). Although it is time consuming to perform and requires more equipment, glassware and consumables, it is more sensitive than the Membrane Filtration.

Aims and objectives

This study aims at locating the various sources of drinking sachet water in Umuahia Metropolis and investigating the Bacteriological quality of these water sources for the presence of bacteria (pathogens).

Therefore the objectives of this research are to:

- (1) Relate our findings to Sanitation, Inadequate disposal of sewage and water-borne infections and diseases;
- (2) Create awareness as to the dangers of drinking unsafe water.

Materials and Methods

Sample collection

Twenty (20) Samples of water were collected by standard methods (WHO, 1995; Cheesbrough, 2000) using heat sterilized bottles with screw caps.

Twenty (20) sachet water packs were bought from different hawkers in the city.

Materials and reagents

Methods

The samples were analysed in less than 5 hours after collection: between 9.00 am and 12.00 noon. The Most Probable Number (MPN) method was used to analyse the 20 water samples (20 Sachet Waters).

Presumptive Coliform Test

The water samples were mixed thoroughly and 50 ml amount poured directly into the 50 ml sterile broth (Double strength) by making up to 100 ml mark previously made on the bottle.

A 10 ml sterile hypodermic syringe (with needle, each) was used to inoculate each 10 ml sterile MacConkey's broth (MCB), double strength. While 2 ml and 0.2 ml (immunization syringes and needles) each was used to inoculate the 10 ml (single strength) and 5 ml (single strength) sterile broth respectively - see Table 7.

Incubation

The seeded bottles were incubated aerobically (with the bottles loosely capped) at 37°C for 24 hours and 48 h. At the end of 48 h, the broth cultures that showed production of acid (colour change from purple to yellow) and gas (seen in the Durham tube) were considered positive for "Presumptive coliform count". Bottles that failed to produce gas and acid at the end of 48 h incubation were presumed to contain no coliform bacteria (recorded as 0 or zero).

Determination of Most Probable Number (MPN)

From the various combinations of the sample bottles that were both positive and negative, results were deduced.

Confirmatory Coliform Tests

MacConkey Broth (MCB), single strength (S/S) was used in place of Brilliant Green Lactose (bile) Broth (BGLB) for confirmatory Coliform (Eijkman) Test.

Using a sterile wire loop, a loopful was subcultured from each presumptive positive broth culture into bottles containing respectively sterile MCB and Tryptone water and incubated for 24 h at 44°C aerobically in waterbath (with the bottles loosely capped). The positive samples (with gas production were recorded as before).

Isolation of organisms

Subculturing On Solid Media

Positive broth cultures were subcultured on the various solid media (BA, CLED, MCA, TSI) for isolation of implicated organisms. These were incubated aerobically and anaerobically (with increased at 37°C overnight). The overnight culture plates were identified morphologically (by colonial appearance), Gram's reactions and Biochemical/ enzyme reaction and sugar fermentation test.

Gram's Staining

Using a sterile (flamed to red-hot and cooled) wire loop, a loopful of the 48 h MCB broth culture was dropped onto a clean, grease-free glass slide and made into a smear. In addition, a colony was picked from the solid culture media, and smears made, using sterile normal saline as well. These were air-dried, fixed with gentle heat and stained by Gram's method (Baker and Silverton, 1976). Slides were examined with x 100 (oil immersion objective).

Biochemical Test

Rosco Tablets (commercially prepared) were used for most tests by simply adding to a saline suspension of the test organism (0.25 ml + an inoculum in about 0.5 mg tablets) in a small test tube, and the test tubes incubated at 37°C and read overnight see Table 18.

Catalase Test

Hydrogen peroxide, 3% H₂O₂ (10 volume solution as used according to Cheesbrough (2000)

Other test

Oxidase, Coagulase, Motility, Urease tests were carried out according to Cheesbrough (2000).

The Triple sugar iron agar slopes (in tubes) were heavily streaked over the surface of the slope, and stab-inoculated into the butt (Mackie and McCartney, 1996). These were incubated aerobically at 37°C overnight.

Yellow slope and a yellow butt indicate the fermentation of lactose and possibly glucose due to *E. coli* and *Enterobacter*; while Pink-red (alkaline reaction) and yellow butt (acid reaction) with small blackening due to H₂S production along the stab is typical of *Salmonella typhi*.

Indole Test

A drop (about 0.1 ml) of Kovac's reagent was added to each tube of the tryptone water culture (48 h old) and gently mixed. A red colour in the surface layer (indole positive) confirmed *E. coli*.

Motility Test

About 10 ml of semi-solid Nutrient Agar (NA) containing about 0.2% agar was dispensed into a test tube and left in a vertical position. A single stab was made with an inoculum down the center of the tube to about half the depth of the medium (using a straight wire). This was incubated at 37°C, and examined after 6 hours and overnight incubation.

Growth of a non-motile organism was restricted to the stab line in the semi-solid nutrient agar. A diffuse growth, or "swarm", of a motile organism was seen extending as a zone of turbidity from the stab line.

Results

Table1 : Results of Contamination of different Satchet water Sources

S/No	Organisms Identified	Samples from 20 Satchet water
1.	<i>Escherichia coli</i>	4 (20%)
2.	<i>Streptococcus</i> spp	2 (10%)
3.	<i>Pseudomonas</i> spp	Nil (0%)
4.	<i>Enterobacter</i> spp	Nil (0%)
5.	<i>Bacillus</i> spp	1 (5%)
6.	<i>Salmonella</i> spp	Nil (0%)
7.	Fungi	Nil (0%)
8.	<i>Proteus</i> spp	Nil (0%)
9.	Mixed contamination	Nil (0%)
10.	No Growth (NG)	13 (65%)

Discussion

All water samples analyzed gave confirmatory coliform test results of between <1 - 18 organisms/100 ml (0 - 18 org/100 ml water). It is virtually impossible to obtain drinking water of 100% purity in any naturally occurring sources of water, according to Chave and Jackson (1999), but a city water supply habouring 4(20%) SW water is an indication of poor hygiene and sanitation, and a general collapse in the provision of safe drinking water. The *E. coli*, with the other isolated organisms (*Streptococcus*, *Enterobacter*, etc) may not actually constitute a public health hazard in a population of healthy individuals, but their presence is a pointer to the presence of or an indication that pathogenic bacteria, viruses, parasites, protozoa, including other aquatic microorganisms that may cause diarrhoea, typhoid and paratyphoid fevers, cholera and dysentery are also present. Incidentally all the isolates: *E. coli*, *Streptococcus* spp, *Klebsiella* spp, *Enterobacter* spp, *Bacillus* spp, *Salmonella* and *Proteus* spp may at one time or the other be found in the intestinal tract of man which strongly confirms the fact that the water sources in Umuahia are mainly polluted by faecal matter.

The samples were collected and analyzed during the rainy season. The result therefore corroborates the work of Kravitz et al. (1990) that potability of water would be expected to deteriorate during the rainy months, because bacterial contamination of ground water generally increases after heavy rains due to eutrophication. Consequently, the spreading of slurry has been shown to have caused contamination of drinking water supply resulting in a small outbreak of water-borne diseases (Watkins and Cameron, 1991).

"Guidelines for drinking water quality" by WHO (1997) reiterates this fact that water quality may deteriorate in the distribution system due to ingress and contamination or the growth of organisms which can take advantage of elevated temperatures and organic nutrients. Also, that change in climate and rainfall will result in changes in the microbiological quality of water, both raw and in distribution. The growth of organisms such as coliforms and aeromonads may be seen more frequently. But Umolu and Aemere (2001) disagree with Kravitz et al. (1990), and argue that high faecal coliform is higher in the dry season, which they attribute to excessive evaporation of water and temperature increase and concentration of the bacterial population in the water.

While *Vibrio*, *Mycobacterium*, *Clostridium*, *Leptospira* and *Yersinia* spp may be present in waste water, their concentration is usually too low to initiate disease outbreaks. The fact remains that the presence of *E. coli* in such high load of up to 58% of drinking water samples informs of recent (and perhaps continuous) contamination. Since enteric bacteria do not multiply in water, rather, they die off easily leaving the saprophytic and other "hardy" bacteria, this may reveal a great deal of continuous contamination of water sources. It is noteworthy that *Salmonella*, a water-borne pathogen (like a few other bacteria) is difficult to culture from water due to its ability to enter into a viable, but not culturable state after exposure to oligotrophic, aquatic environment (EPA/625/R-92/004, 1992). Yet, our investigation revealed the presence of *Salmonella* spp in 1(5%) of SW. This gives a warning signal as to the rate of incessant typhoid fever that is regularly being reported in our clinics.

In most countries the principal risks to human health associated with the consumption of polluted water are microbiological in nature (although the importance of chemical contamination should not be underestimated). An estimated 80% of all diseases and over one-third of deaths in developing countries are caused by water related diseases. In drawing up standards for drinking water quality, it will be necessary to take into account various local, geographical, socioeconomic and cultural factors.

Conclusion

Our investigations revealed that Public water supply of tap water in Umuahia (through the Umuopara Water Scheme) is most times not treated at all. And supplies, when available, are intermittent; which leaves consumers at the mercy of private entrepreneurs who sink the boreholes, whose supervision by the appropriate government authorities is suspect. They resort to the use of satchet water with hope that they are safe drinking water. The study revealed that some of the satchet waters are contaminated because of the way and manner they are prepared. Those living in Umuahia should take hygiene seriously to avoid some diseases.

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