

Microbiological Quality of Natural Spring Water from Selected Villages in Enugu and Ebonyi State, South/East Nigeria

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Abstract: The assessment of natural spring water from Igbo-Etiti, Amakporo and Nsokkara was designed to determine the microbiological quality of natural spring waters mainly used for drinking at various communities of Enugu and Ebonyi state of south/Eastern Nigeria. Membrane filtration method was used for the study. Water samples were collected morning, afternoon and evening, homogenized and analyzed. The water borne microorganisms were characterized and identified after incubation at 30°C temperature through morphological and biochemical test methods; they were confirmed using standard microbiological manual. The characterization and identification implicated *Escherichia coli*, *Pseudomonas* sp., *Aeromonas* sp., *Vibrio* sp., *Proteus* sp., *Klebsiella* sp., *Shigella* sp., *Streptococcus* sp. and *Staphylococcus* sp. as major microbial contaminants. The colony forming unit (CFU), was determined with *Escherichia coli* implicated to show the highest number of colonies and frequency of occurrence, followed by *Pseudomonas* sp., *Proteus* sp., *Shigella* and *Aeromonas* sp. The least occurrence featured *Vibrio* sp., *Staphylococcus* and *Streptococcus* sp. The study revealed that the samples from Igbo-Etiti natural spring water, was heavily contaminated with pathogenic bacteria, also, health threatening microorganisms like *Vibrio* and *Shigella* sp. This equally revealed that all the natural spring waters analyzed were heavily contaminated with fecal coliform bacteria, showing that the water from these sources are unfit for drinking and domestic use; if not properly boiled and filtered.

Key words: Natural Spring Water • Isolation • Coliform • Bacteria • Assessment

INTRODUCTION

The second most important requirement of man is water. Water is required for proper physiological function of living things including man. The average water intake required by man per day is about 3.7 liters for male (15 cups) and 2.7 liters for female (11 cups). Despite the abundant bodies of water in nature, the availability of portable water is a global issue that has not been overcome completely by any country of the world. Though human body requires up to 3.7 liters of water per day for proper function, yet water on the other hand can constitute nuisance to human health when consumed without screening for the presence of pathogenic microorganisms [1]. The water supply system in the south-Eastern part of Nigeria is erratic in nature and does

not cover most of the local Government Area or communities, but few streets in the capital cities of the states. The economic situation of the country has adjusted the life of the teeming population of the common man to resort to consuming any available water within their disposal, just to keep life going. Because of government inability to provide water for the citizens, many families who can afford motorized borehole are comfortable with their private source of water while the teeming population of less privilege ones makes use of available natural spring waters located at different positions in their communities by nature. Just as in Ebonyi and Enugu State of Nigeria. There is a serious threat of international and inter-community disputes over water supplies, calling for communal management and supply their available water in order to overcome the

challenges of water availability and distribution [2]. The portability of household table water is necessary to protect the life of the consumers [3]. Research has shown that most unprotected water sources such as springs, traditional wells and ponds, can be improved and may be preferred to constructing a new motorized borehole. However, unprotected sources are open to contamination and pose a potential health risk [2].

The mortality of water associated diseases is currently above five million people per year [2]. It has been observed that more than 50% are of microbial origin, with cholera standing out [4]. Waste water discharge in fresh waters and coastal waters are the major source of fecal contamination of water [5, 7]. Traditionally, ground water had been considered the least water source to be contaminated by human or animal waste, most especially ground water from the deep, confined aquifers. It is assumed that water passes through the soil filters off most impurities and microorganisms, therefore, leaving the consumers with little concern of ground water contamination [8]. Despite that spring waters are considered aesthetically acceptable for domestic uses, poorly designed pit latrines, poor waste water management as well as inadequate spring management may lead to microbial contamination [9]. Some microorganisms of concern in water contamination include *Salmonella* sp., *Shigella* sp., *Escherichia coli* and *Vibrio cholera* [10]. The presence of *E. coli* is an indication of the presence of any of the other water borne pathogens. Table water qualities include: odourless, colourless, tasteless, soft, free from fecal contamination and harmful chemicals [2]. The microbiological examination of water is used naturally and scientifically worldwide to monitor and control the quality and safety of drinking waters [11].

Aim: This investigation is aimed at assessing the microbiological quality of some selected natural spring water sources located in various villages in the both Enugu and Ebonyi State, serving as major sources of drinking water.

MATERIALS AND METHODS

This study was carried out in two states of South/East geopolitical zone of Nigeria, covering Enugu and Ebonyi State. These include; Ase, Ujere and Atturu natural spring water in Igbo-Etiti Local Government Area of Enugu State, Barara natural spring water in ObunoAmakporo in Onicha local Government and Nsokkara, Echara and amuzu natural spring water in Nsokkara Community in Ezza South Local Government Area of Ebonyi State.

Sample Collection: The spring water samples were collected from Ujere, Ase and Atturu natural spring waters in Igbo-Etiti local Government Area of Enugu state, Barara natural spring water in Obuno Amakporo in Onicha Local Government Area of Ebonyi State and Nsokkara, Echara and Amuzu natural spring water in Nsokkara Community in Ezza south Local Government Area, Ebonyi state all in South/Eastern Nigeria. The Samples were collected aseptically with 100ml capacity bottle in duplicate. All the samples were collected between 6 am and 8 am when little children have not had access to these outstanding sources of drinking water for the communities. The samples were transported to the laboratory in ice box for analysis [21].

Sample Preparation: In the membrane filtration method, a 100 ml water sample was vacuumed through a filter using a vacuumed pump. After filtration, the bacteria remain on the filter paper was placed in a Petri dish containing nutrient broth. The Petri-dishes were placed in an incubator at 37°C for 18-24 hours to accommodate both total and fecal coliform bacteria [12]. After incubation, the bacteria colonies were observed with the naked eye and recorded appropriately.

Preparation of Stock Culture: The observed mixed cultures were sub-cultured severally, to obtain a pure isolates. This was carried out by transferring a loop full of each colony to nutrient agars slant and incubated at room temperature for 24 hours. The isolated organisms were stored at 4°C [12, 20].

Gram Stain: Gram staining distinguishes nearly all bacteria as Gram positive or Gram negative according to whether they resist decolorization of crystal violet by acetone or not [12, 18, 19].

Catalase Test: This demonstrates the presences of catalase, an enzyme that catalyze the release of oxygen from hydrogen peroxide. It is useful in differentiating aerobic organisms from closely related but anaerobic species [12].

Oxidase Test: This test was carried out to screen smears of oxidase positive organisms such as *Neisseria*, *Alcaligenes*, *Aeromonas* and *Pseudomonas* sp., from oxidase negative organism like *Enterobacteriaceae* family [12].

Indole Test: This test was done according to the method prescribed by Cheesbrough [12] and observed for the production of indole from tryptophan, which was supplied by the peptone water broth.

Table 1: Considerable Morphological and Biochemical Characteristics of The Isolates form Igbo-Etiti L.G.A. of Enugu, Amakporo, Onicha L.G.A. and Nsokkara ezza South L.G.A. Both Ebonyi State

Sample name	CFU/10ml	CFU %	Morph	G.R	TCBS	EMB	Motility	cat	Cit	ox	Indo	lacto	Glu	Suspected organism
Igbo-Etiti	4	3.3	Yellow	-c	Y	-	+	+	D	+	+	-	A/G	<i>Vibrio</i> sp.
	13	10.8	Yellow	-R	Y	D	-	-	-	+	+	D	-	<i>Aeromonas</i> sp.
	31	25.8	Green	-R	-	Pink	+	+	+	-	+	-	A	<i>Pseudomonas</i> sp.
	43	35.8	Grayish	-R	-	Pink	+	+	-	-	+	+	A/G	<i>Escherichia coli</i>
	8	6.6	White	-R	-	Pink	+	+	-	-	+	-	A/G	<i>Proteus</i> sp.
	7	5.8	White	-R	-	White	-	+	-	-	+	-	A/G	<i>Shigella</i> sp.
	4	3.3	Mucoid	+ci	-	-	-	+	+	-	-	+	-	<i>Staphylococcus</i> sp.
	10	8.3	Mucoid	+ci	-	-	+	-	-	+	+	-	A/G	<i>Streptococcus</i> sp.
	120													
Sample name	CFU/10ml		Morph	G.R	TCBS	EMB	Motility	cat	Cit	ox	Indo	lacto	glu	Suspected organism
Amakporo	5	4	White	-R	-	White	-	+	-	-	+	-	A/G	<i>Shigella</i> sp.
	58	46	S	-R	-	Pink	+	+	-	-	+	+	A/G	<i>Escherichia coli</i>
	42	33.3	G	-R	-	Pink	+	+	+	-	+	-	A	<i>Pseudomonas</i> sp.
	21	16.7		-R	Y	Purple	-	+	-	-	-	+	+	<i>Klebsiella</i> sp.
	126													
Sample name	CFU/10ml		Morph	G.R	TCBS	EMB	Motility	cat	Cit	ox	Indo	lacto	glu	Suspected organism
Nsokkara	24	26.6			Y	Purple	-	+	-	-	-	+	+	<i>Klebsiella</i>
	41	45.6	S	-R	-	Pink	+	+	-	-	+	+	A/G	<i>Escherichia coli</i>
	17	18.9	G	-R	-	Pink	+	+	+	-	+	-	A	<i>Pseudomonas</i> sp.
	8	8.9	White	-R	-	Pink	+	+	-	-	+	-	A/G	<i>Proteus</i> sp.
	90													

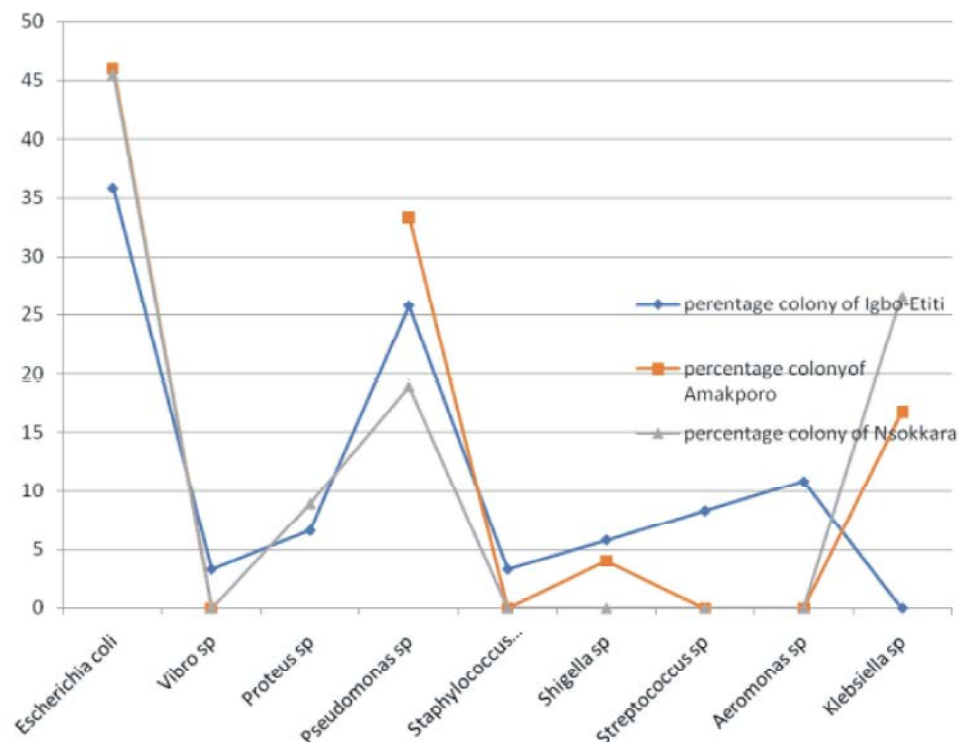


Fig. 1: Frequency of occurrence of the isolate from different sample sources

Fermentation of Carbohydrate: This refers to the production of acids from sugars, (glucose) glycoside and polyhydric alcohols. A wide variety of carbohydrates are fermented by bacteria and the pattern of fermentation is a characteristic feature of certain species [12].

DISCUSSION

The microbial examination of natural spring waters from Enugu and Ebonyi state was determined and it was observed that *Escherichia coli*, *Pseudomonas* sp.,

Aeromonas sp., *Vibrio* sp., *Proteus* sp., *Klebsiella* sp., *Shigella* sp., *Streptococcus* sp. and *Staphylococcus* sp. was isolated from the various sampled bodies of water and of varying percentage of frequency of occurrence in the different bodies of the natural spring waters sampled. The natural spring water of Ujere, Ase and Aturu in Igbo-Etiti Local Government Area of Enugu State contained *E.coli*, *Vibrio* sp., *Aeromonas* sp., *Pseudomonas* sp., *Shigella* sp., *Proteus* sp., *Staphylococcus* and *Streptococcus* sp. From Amakporo Onicha natural spring water, the following organisms were implicated, they include: *E.coli*, *Pseudomonas* sp., *Shigella* sp., *Klebsiella* sp. while Nsokkara, Echara and Amuzu in Ezza North was isolated *E.coli*, *Pseudomonas* sp, *Proteus* sp, *Klebsiella* sp.

The microbial analysis of Ujere, Ase and Aturu in Igbo-Etiti showed that *E. coli* had the highest number of colonies (43CFU/10ml) with the percentage of 35.8% of the total colony count, *Pseudomonas* was the next organism with a very high number of colonies (31CFU/10ml) with 25.8% of total colony, followed by *Aeromonas* with 13 CFU/10ml and 10.8%, *Proteus* had 8 CFU/10ml with 6.6% of the colony count, *Shigella* had 7 CFU/10ml with 5.8% of the total bacteria colony *Vibriosp* had the least of the Gram negative bacteria isolated with the colony count of 4CFU/10ml and 3.3% of the total count. Among Gram positive bacteria organisms isolated are *Streptococcus* sp with number of colony 10 CFU/10ml and 8.3% of total number of bacteria colony, followed by *Staphylococcus* sp with 4 CFU/10ml and 3.3% of the total bacteria isolated from Igbo-Etiti natural spring water in Enugu State.

The microbial examination result of Amakporo natural spring water revealed that *E. coli* was the leading cause of contamination with 58 CFU/10ml and 46% of the total colony, followed by *pseudomonas* with 42 CFU/10ml and 33.3% total colony, *Klebsiella* had 21CFU/10ml and 16.7% of the total colony and the least was *Shigella* which had 5 CFU/10ml and 4% of the total bacteria colony isolated from Amakporo in Onicha Local Government Area. Nsokkara revealed that *E. coli* was the leading cause of contamination in the number of bacteria colony count, showed 41CFU/10ml and 45.6% of the total colony count, followed by *Klebsiella* sp with 24 CFU/10ml and 26.6% of total bacteria colonies, *Pseudomonas* had 17 CFU/10ml with 18.9% of the total colony count with the *Proteus* showed the least number of colony of 8CFU/10ml and 8.9% of the total bacteria colony count isolated from Nsokkara in Ezza Local Government Ebonyi State.

The analysis of microbial contamination of natural spring waters used as the most accessible source of drinking and domestic water in the two neighboring states Enugu and Ebonyi revealed that the water sources are contaminated with microorganisms. The total coliforms are known to be bacteria mostly found in the environment, such as soil, vegetation, as well as the intestine of mammals including humans. These organisms are not likely to cause illness, but their presence is a clear indication of the vulnerability of the water to recent fecal contamination, likewise the possibility of presence of disease causing pathogens. Although, a particular strains of *E. coli*, particularly the strain 0157:H7, can cause serious illness as other pathogenic organisms [13]. The result above revealed that *Escherichia coli* had the highest microbial load and frequency of occurrence in all the water sampled; that is to say that the water is not in conformity with WHO's standard of every 100 mL of drinking water tested, no total coliforms or *E. coli* should be detected [2, 22] and UNEP/WHO [14]. The presence of *Staphylococcus* sp and *Streptococcus* sp in the natural spring water from Igbo-Etiti as shown in table 1 above agrees with the work of Edema *et al.* 2001 who reported the presence of *Staphylococcus* sp and *Streptococcus* sp in natural spring water from Abeokuta [15]. The result also agrees with the work of Kalu and Doris [16] and Chidinma *et al.* [17] who reported the presence of both Gram positive and negative bacteria with *Escherichia coli* more in abundance in Assessment of Streams used for drinking in Afikpo North L. G. A. and the presence of *Escherichia coli*, *Pseudomonas* sp, *Klebsiella* and *Staphylococcus* sp from borehole sources in Abakaliki metropolis respectively.

The results of this study showed some level of microbial contamination which varies in frequency of occurrence. The isolated microorganisms have been shown to be among some common bacteria organisms found in water. Fig. 1 above showed that *Escherichia coli* had the highest level of contamination in all the bodies of natural spring water sampled in both states, followed by *Pseudomonas* sp., *Klebsiella* sp. while *Vibrio* sp., *Staphylococcus* sp., *Streptococcus* and *Aeromonas* sp. had the least level of contamination.

CONCLUSIONS

With the above result shown that the natural spring water sources in this study area are not fit for drinking, it is of great importance that an alternative source of water

is provided to the residence of the communities. Otherwise, the water should be boiled before drinking, using in preparing infant formulas, preparing juices and ice cubes, brushing of teeth, washing of fruits and vegetables. This will go a long way in curbing the proliferation of waterborne diseases like Cholera, Diarrhea, Dysentery, Typhoid Fever and Hepatitis A.

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