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## Microbiological quality of Natural Spring Waters in Enugu and Ebonyi state, south/East Nigeria.

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### ABSTRACT

The assessment of natural spring water from Igbo-Etiti, Amakporo and Nsokkarawas designed to determine the microbiological quality of natural spring waters located 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 temperature of 30°C, through morphological and biochemical test methods, and confirmed using standard microbiological manual. The characterization and identification showed the presence of *Escherichia coli*, *Pseudomonas spp*, *Aeromonas spp*, *Vibrio spp*, *Proteus spp*, *Klebsiella spp*, *Shigella spp*, *Streptococcus spp* and *Staphylococcus spp* as major microbial contaminants. The colony forming unit (cfu) was determined, with Igbo-Etiti showing to show the highest number of colonies (24), followed by Amakporo 12 colonies and the least Nsokkarawa with 11 colonies. *Escherichia coli* was found to have the highest frequency of occurrence, followed by *Pseudomonas spp*, *Klebsiella spp*, *Shigella spp*, *Proteus spp*, *Aeromonas spp* and *Streptococcus spp*. The least occurrence featured *Vibrio* and *Staphylococcus spp*. The study revealed that the samples from Igbo-Etiti natural spring water, was heavily contaminated with pathogenic and health threatening microorganisms like *Vibrio* and *Shigella spp*. This equally revealed that all the natural spring waters analyzed were all heavily contaminated with fecal coliform bacteria showing that the analyzed waters were unsafe for drinking as well as domestic purposes which include: washing of hands, plates and fruits if not properly boiled and filtered.

**Keywords:** Pathogenic, Isolation, Coliform, Contamination and Assessment.

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### 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 nature 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, only few streets in the capital cities of the states have accessible drinking water. 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 whereas the teeming population of less privileged ones makes use of available natural spring waters located at different positions in their communities by nature, this scenario is 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 to 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 [4].

The mortality of water associated diseases is currently above five million people per year [5]. It has been observed that more than 50% are of microbial origin, with cholera standing out, [3]. Waste water discharge in fresh waters and coastal waters are the major source of fecal contamination of water [6],[7] and [8]. 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, passing through the soil, filters off most impurities and microorganisms, therefore, leaving the consumers with little concern of ground water contamination<sup>[14]</sup>. 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 indicator, on 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 [11]. The microbiological examination of water is used naturally and scientifically worldwide to monitor and control the quality and safety of drinking waters [12].

## 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 Obunoma Amakporo 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 ObunoAmakporo in Onicha Local Government Area of Ebonyi State, and Nsokkara, Echara and Amuzunatural spring water in Nsokkara Community in Ezza south Local Government Area, Ebonyi state all in South/Eastern Nigeria.

#### **Sample preparation**

Samples were filtered thoroughly with membrane filter (Nalgene, India). 100ml each of the samples was poured into the membrane filter with already fixed filter paper of 0.45µm, batch number Rs. 3483, manufactured by Millipore (India) Pvt Ltd. After filtration, the filterpaper was placed in a Petri dish containing nutrient broth. The Petridishes were placed in an incubator at 37°C for 18-24 hours to accommodate both fecal and other coliform bacteria. After incubation, the bacteria colonies were observed with the naked eye and recorded appropriately.

#### **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,[13].

**Oxidase test:** This test was carried out to screen smears of oxidase positive organisms such as *Neisseria*, *Alcaligenes*, *Aeromonas* and *Pseudomonas* spp, from oxidase negative organism like *Enterobacteriaceae* family according to [6]

The Samples were collected aseptically with 100ml capacity bottle in duplicate. All the samples were collected and stored in the cold box. The samples were transported to the laboratory in cold box for analysis.

**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 (Cheesbrough, 2006).

**Gram stain:** Gram staining distinguishes nearly all bacteria as Gram positive or Gram negative according to whether they resist decolourization of crystal violet by acetone or not. According to Cheesbrough (2006).

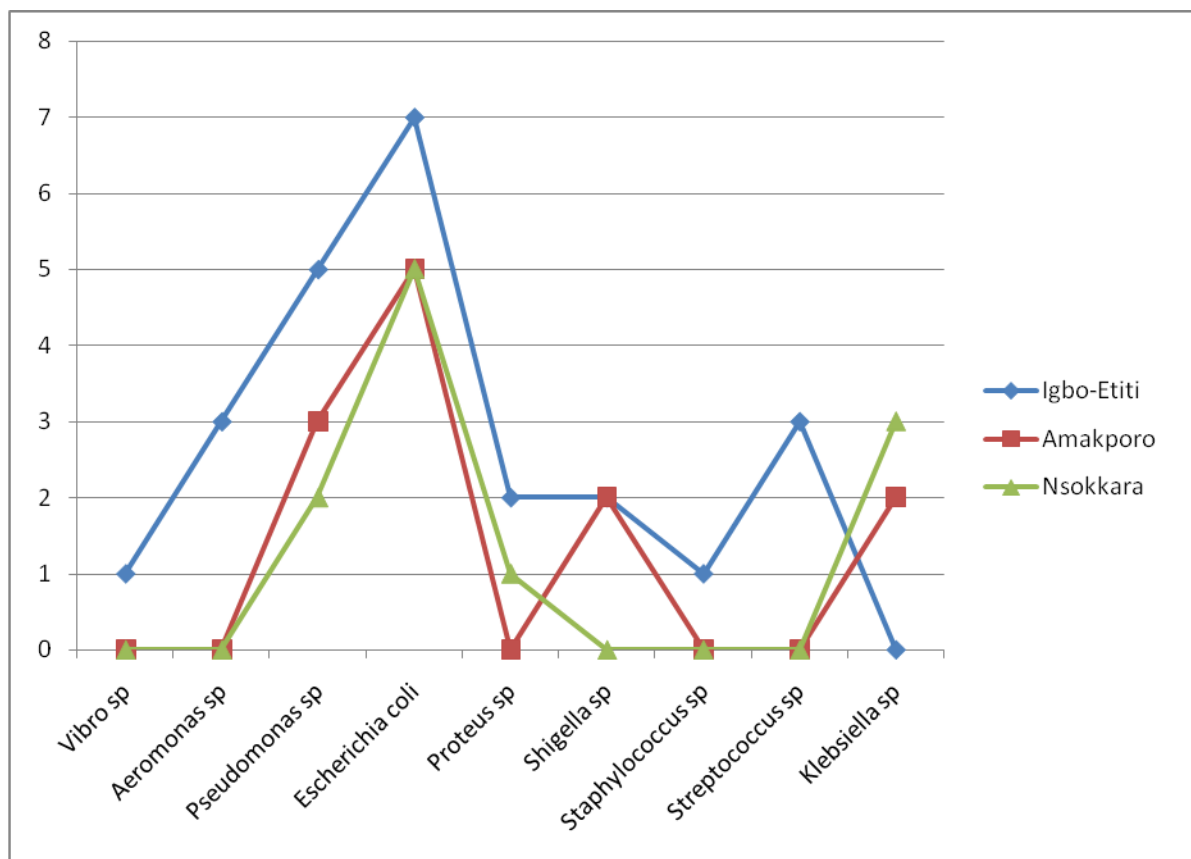
**Indole test:** This test was done according to the method prescribed by [6] and observed for the production of indole from tryptophan, which was supplied by the peptone water broth.

**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 [14].

Table1 below showed 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 States

Sample name/ total CFU	Frequency of occurrence	Percentage occurrence (%)	G.R	TCB S	EMB	motility	Cat	Cit	ox	Ind o	lacto	Glu	Suspected organism
Igbo-Etiti 24	1	4	-c	Y	-	+	+	D	+	+	-	A/G	<i>Vibrio</i> sp
	3	13	-R	Y	D	-	-	-	+	+	D	-	<i>Aeromonas</i> sp
	5	21	-R	-	Pink	+	+	+	-	+	-	A	<i>Pseudomonas</i> sp
	7	29	-R	-	Pink	+	+	-	-	+	+	A/G	<i>Escherichia coli</i>
	2	8	-R	-	Pink	+	+	-	-	+	-	A/G	<i>Proteus</i> sp
	2	8	-R	-	Pink	-	+	-	-	+	-	A/G	<i>Shigella</i> sp
	1	4	+ci	-	-	-	+	+	-	-	+	-	<i>Staphylococcus</i> sp
	3	13	+ci	-	-	+	-	-	+	+	-	A/G	<i>Streptococcus</i> sp
Sample name/ total CFU	Frequency of occurrence	Percentage occurrence (%)	G.R	TCB S	EMB	motility	cat	Cit	ox	Ind o	lacto	glu	Suspected organism
Amakporo 12	2	16.5	-R	-	Pink	-	+	-	-	+	-	A/G	<i>Shigella</i> sp
	5	42	-R	-	Pink	+	+	-	-	+	+	A/G	<i>Escherichia coli</i>
	3	25	-R	-	Pink	+	+	+	-	+	-	A	<i>Pseudomonas</i> sp
	2	16.5	-R	Y	Purple	-	+	-	-	-	+	+	<i>Klebsiella</i> sp
Sample name/ total CFU	Frequency of occurrence	Percentage occurrence (%)	G.R	TCB S	EMB	motility	cat	Cit	ox	Ind o	lacto	glu	Suspected organism
Nsokkara 11	3	27		Y	Purple	-	+	-	-	-	+	+	<i>Klebsiella</i>
	5	46	-R	-	Pink	+	+	-	-	+	+	A/G	<i>Escherichia coli</i>
	2	18	-R	-	Pink	+	+	+	-	+	-	A	<i>Pseudomonas</i> sp
	1	9	-R	-	Pink	+	+	+	+	+	-	A/G	<i>Proteus</i> sp

Figure 1 below showed the frequency of occurrence of the isolate from different sample sources.



## Discussion

The microbial examination of natural spring waters from Enugu and Ebonyi state was determined and it was observed that *Escherichiacoli*, *Pseudomonasspp*, *Aeromonasspp*, *Vibriosspp*, *Proteusspp*, *Klebsiellasp*, *Shigellasp*, *Streptococcusspp* and *Staphylococcusspp* was isolated from the various sampled bodies of water and of varying 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*, *Vibriosspp*, *Aeromonasspp*, *Pseudomonasspp*, *Shigellasp*, *Proteusspp*, *Staphylococcus* and *Streptococcus spp*. From Amakporo Onicha natural spring water, the following organisms were implicated, which include: *E.coli*,

*Pseudomonasspp*, *Shigellasp*, *Klebsiellasp* while Nsokkara, Echara and Amuzu in Ezza North was isolated *E.coli*, *Pseudomonasspp*, *Proteusspp*, *Klebsiella spp*.

The microbial analysis of Ujere, Ase and Aturu in Igbo-Etiti showed that *E. coli* had the highest number of occurrence of 7(29%), *Pseudomonas* was the next organism with a very high number of occurrence 5(21%), followed by *Aeromonas* and *Streptococcus* with 3(8%) each, *Shigella* and *Proteus* had 2(8%) each and lastly, *Staphylococcus* and *Vibriosspp* had the least number of occurrence of 1(4%) each. Among Gram positive bacteria organisms isolated are *Streptococcusspp* and *Staphylococcusspp* other were Gram negative bacteria.

The microbial examination result of Amakporo natural spring water revealed

that *E.coli* was the leading cause of contamination with 5 number of occurrences of 42%, followed by *pseudomonas* with 3 number of occurrences of 25%, while *Klebsiella* and *Shigella* had 2 number of occurrences of 16.5% each. Nsokkara revealed that *E. coli* was the leading cause of contamination in the samples with 5 number of occurrences making 46%, followed by *Klebsiella* spp with 3 frequencies of occurrence with 27%, *Pseudomonas* occurred two times with, while the least was *Proteus* which appeared once and had 9%.

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 count was determined, likewise bacterial count. Mostly organisms isolated were from environmental and fecal origins. The presence of these organisms in the major source of drinking water is a serious threat to the life of consumers. The number of *Escherichiacoli* in drinking water should be zero, it is expected that drinking water should have passed through one form of treatment or the other. The result above revealed that *Escherichiacoli* had the highest number of occurrence in all the water sampled; that is to say that the water is not in conformity with WHO's standard for drinking water according to UNEP/WHO 1996. The presence of *Staphylococcus* spp and *Streptococcus* spp in the natural spring water from Igbo-Etiti as shown in table 1 above agreed with the work of [5], who reported the presence of *Staphylococcus* spp and *Streptococcus* spp in natural spring water from Abeokuta. The result also agreed with the work of [3] and [15], who reported the presence of both Gram positive and negative bacteria with *Escherichia coli* leading in the frequency of occurrence in "Assessment of Streams used for drinking in Afikpo North L. G. A. and the presence of *Escherichia coli*, *Pseudomonas* spp, *Klebsiella* and *Staphylococcus* spp from

borehole sources in abakaliki metropolis respectively".

The result 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. The 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* spp, *Klebsiella* spp while *Vibrio* and *Staphylococcus* spp, had the least level of contamination.

With the above result of this research, the natural spring water sources in these study areas are not fit for drinking, untreated. It is of great importance that treatment of these water must be done before consumption or used domestically. On the other hand, an alternative source of water domestic water should be provided to the residence of the communities for safety of lives, mostly children. Otherwise, adequate sensitization on how to boil and filter water should be given to the communities using the natural spring waters as their source of drinking water; 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 in the rural areas.

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