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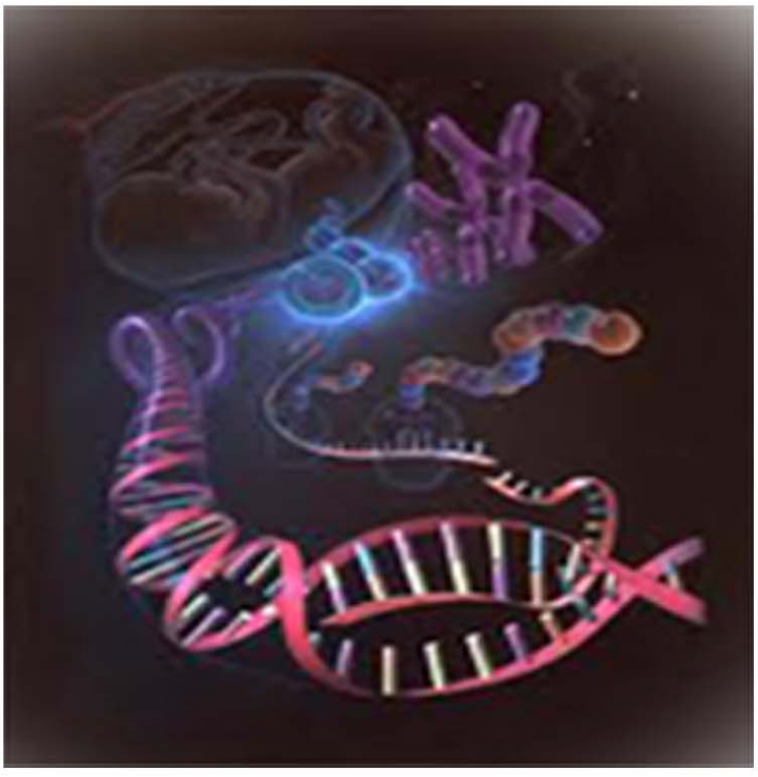

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Research Paper

BACTERIOLOGICAL ANALYSIS OF PIPE-BORNE WATER AND WELL WATER WITHIN ENUGU EAST, ENUGU STATE NIGERIA

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The bacteriological examination of water sourced within Enugu East was carried out to determine the bacterial load in the water samples and to determine the organisms that contaminate it. The water samples were pipe-borne water and well water. They were collected within Enugu East in sterile containers. Using pour plate plating technique 0.1ml of water sample was pipetted into petri dishes and already prepared agar (Nutrient agar and MacConkey agar) were poured into the Peri-dishes, swirled for a homogeneous mixture, allowed to solidify and incubated at 37°C for 24 hrs. Presumption coliform counts were carried out by using double strength lactose and single strength lactose broth in test tubes and these were incubated at 37°C for 24°hrs. The result showed high counts of microorganism in well water while pipe-borne water had low counts. The bacterial isolated are *Citrobacter spp., Escherichia coli* and *Klebsiella Spp.* These showed that well water contained contaminants and there should be need for adequate treatment of water to avoid water borne diseases such as Cholera, Typhoid Fever, Dysentery and *Escherichia coli* infection.

Keywords: *Citrobacter spp., Escherichia coli, Klebsiella Spp.,* Bacteriological analysis and Coliform counts.

INTRODUCTION

Bacteriological water analysis is the method of analyzing water in order to determine the bacterial load of the water sample. It is a microbiological analytical procedure which uses water sample to determine the concentration of bacteria in that sample (Henniker, 1997).

Water as proclaimed by economists is an essential commodity for human consumption. Scientifically, water is a chemical substance with the chemical formula H_2O (Bramer, 2011). A water molecule contains one oxygen and two hydrogen atoms connected by covalent bonds. In a Layman's understanding, water is colorless, odourless and tasteless liquid essential for plant, and animal life and it is most widely used as a universal solvent.

Although the earth contains an abundance of water, it is known that only a small percentage of it is fresh water. A smaller amount of this fresh

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water is accessible and usable by humans and animals (Mathew, 2009). As the human population grows rapidly, the amount of freshwater available per person shrinks. The relatively small amount of available free water demonstrates how critical it is for everyone to help maintain clean healthy water sources (Robert, 2010).

Water is literally the source of life on earth. People begin to feel thirsty after a loss of only 1% of body fluids and risk death if fluid loss near 10% (Park, 2002). Water is a basic human right. Without good quality of domestic water supply, man and other living things may eventually die (Joanne, 2000). Much of the ill-health which affects humanity especially in the developing countries can be traced to lack of safe and wholesome water supply that is free from contamination. There can be no state of positive health and well-being without safe water (Gleick, 1996). Man needs water for drinking, cooking, bathing, sewage disposal, irrigation for agriculture, industrial uses and for recreational purposes amongst many other uses. A study in 1990 estimated that more than 1 billion people in developing countries lacked access to safe drinking water (World Health Organization, 1995).

Water is not only a vital environmental factor to all forms of life, but it has also a great role to play in socio-economic development of human population (Hilton *et al.*, 2007).Water of adequate quality is essential for healthy life. Many diseases are associated with contaminated water. Therefore, the aim of this research was to determine the number of bacterial organisms in pipe-borne water and well water within Enugu East, Enugu state of Nigeria and to recommend ways of preventing them.

MATERIALS AND METHODS

Hardwares

Autoclave, Colony counting chamber, Incubator, Inoculating needle, Retort stand, Bunsen burner, Spatula, Weighing balance and Wire loop.

Glass Wares

Beaker, Conical flask, Petri-dish, Pipette, Thermometer and Test-tube.

Reagents

Hydrogen peroxides (H_2O_2), Kava's reagent, Gram's stain, Peptone water, Methyl red, Phenol red, Potassium nitrate

Sterilization of Materials

All glass wares used were thoroughly washed, dried and sterilized in an oven at 180^{o}C for 2h, media were also, sterilized in an autoclave at 120^{o}C for 15mins.

Collection of the Samples

Bearing in mind that the correctness of the deduction from the result depends on the care with which the samples were collected, every precaution was taken during collection to prevent contamination. Samples were collected in sterile bottles of 100ml capacity. One sample of pipe borne water and one sample of well water were collected from protected pipe borne water and well.

Preparation of the Media

The media for culturing were aseptically prepared as when necessary according to the manufacturer's instructions and autoclaved at 121^{o}C for 15mins. After sterilization, the media were dispersed aseptically into sterilized petri-dishes.

Sample Processing

The Presumption Coliform (Multiple Tube)

The medium used for isolation of the coliform organism was lactose broth. Three rows of 3 tubes were taken and in the first row held 10ml of double strength lactose broth while the tubes in the second and third rows contained 5ml of single strength lactose broth, with a sterile pipette, 10ml of the sample was added to each of the test tubes in row 2 and 0.1ml was added to each of these tests in row 3.

After gentle shaking, the tubes with mixed inoculums were incubated at 37°C for 24h. Each of these tubes contained a sterile Durham tubes for indicating gas formation and were tightly plugged with sterile non-absorbent cotton wool. Tube showing acid (colour changed to yellow). And gas formations were recorded as positive. Other tubes that did not show positive results were both incubated and examined. After this time, tubes that did not show acid and gas formation were discarded from the same water sample. Other inoculums were made on nutrient agar plate to know the other microorganisms that are present in the water sample.

Confirmatory Test

All the positive presumptive tubes were those that gave out gas at the end of 24h, incubated at 37°C and were utilized in the confirmatory test for coliform organisms. The positive tubes were sub-cultured into plated eosin methylene blue agar and were incubated for 24h at 37°C for confirmation of the presence of coliform organisms. The plates were examined for typical coliform of *Escherichia coli*. Colonies were button like in appearance and surrounded by a greenish metallic sheen flat with dark and black centers.

Completed Test

The completed test was performed with the colonies got from the confirmed test that showed the characteristics of *Escherichia coli*. Each selected colony was incubated into tubes of lactose broth and also streaked on nutrient agar slant and the tubes were then incubated for 24h to 48h at 35°C-37°C.

Gram Staining

Each representative colony was used to form a smear on a slide. The heat-fixed smear was laid across a staining rack and placed over a sink. Dry smear was flooded with crystal violet and allowed to stand for 1 min, the smear was washed off with water. Lugols iodine was used to flood the smear to remove excess stain. After a minute, it was washed with water and 95% acetone was applied and allowed to stand for 30 mins. Thereafter, the smear was washed with water and flooded with safaranin for 30 seconds. It was washed off after 30 seconds and allowed to air dry. After drying the smear was viewed using oil immersion microscope. Here two colours were observed: gram positive bacteria showed blue black and gram negative bacteria which showed pink or red.

Indole Test

The tryptophan broth was inoculated with the test sample at 37°C for 28 h then 0.5ml of the Kovac's reagent was added, gently agitated and examined for 1 minute. The upper layer of the liquid of the test tube that turned red indicated.

Motility Test

This test was done using the hanging drop method. A drop of the test organisms in a saline suspension was placed on a cover slip. The cover slip was inverted and placed on a cavity slide, this was viewed under the microscope; a sharp darting movement in different directions across the field view of the microscope indicated a positive motility result.

Sugar Test

1.0g each of different types of sugar like glucose, lactose and sucrose were dissolved separately. To each 10ml of peptone water was added in test tube. This was followed by the addition of 2 drops of 0.1% phenol red. Durham tubes were also placed on to the test tubes inverted in order to detect the production of gas and acid. The tubes were autoclaved at 110^{o}C for 15mins. And after cooling, the tubes were inoculated with bacterial culture using sterilized wire loop under aseptic condition. Un-inoculated tubes were used as control and the tubes were then incubated for 48h at 37^{o}C. A colour change to yellow showed acid production and was recorded as positive fermentation.

RESULTS AND DISCUSSION

The result of the analysis as recorded in (Table 1) above showed that well water was recently and constantly being polluted by either human excreta or animal feaces. The pollution could be as a result of runoff from pasture feedlots, septic tank and sewage plants into ground water (Lattimore, 2010). These contribute to the presence of coliform bacteria and other pathogenic organisms such as *Escherichia coli, Citrobacter spp.* and *Klebsiella spp.* which were detected in contaminated water.

All these organisms detected were all gram negatives as shown in (Table 2). Well water is a potentially hazardous source of water within Enugu East, Nigeria and could be the route of transmission of gas, intestinal and other water borne diseases that were commonly reported by the health authorities in the area. Apart from drinking of contaminated water its use for preparation of food, bathing and watering crops may also lead to water borne diseases.

Though, pipe borne water samples for presumptive test, confirmatory test and completed test showed positive results in some cases, which was as a result of the presence of acid and gas (Tables 3, 4 and 5). Coliform may also, contaminate pipe borne water as a result of contaminated pipe or due to inadequate plumbing systems (Roberts, 2010).

Table 1: Total Plate Count of Viable Coliforms at 37^{o}C for Pathogenic Organisms

S. No.	Pipe-borne Water	Well Water
1.	16	32
2.	28	60
3.	8	24
4.	32	16
5.	36	8
6.	20	40
7.	40	36
8.	44	48
9.	12	52
10.	24	20

Table 2: Morphological Characteristics, Gram Reaction and Biochemical Tests for Isolated Bacteria

Morphological Characteristics	Gram reaction	Indole	Motility	Glucose	Lactose	Sucrose	Organism Isolated
Round raised yellow smooth colonies	-veRods	+	+	AG	AG	G	*Citrobacter spp*
Pink colonies on MacConkey	-veRods	+	+	AG	AG	A	*Escherichia coli*
Pale colonies on Mac Conky agar	-veRods	+	+	AG	AG	AG	*Klebsiella spp*

KEY: A - Acid; B - Gas; AG - Acid and Gas; -Ve - Gram Negative.

Table 3: Presumptive Test (MPN)

S. No.	Pipe-Borne Water	Well Water
1.	AG	AG
2.	AG	AG
3.	-	A
4.	G	A
5.	G	G
6.	A	-
7.	AG	G
8.	A	AG
9.	A	AG
10.	-	A

KEY: A - Acid; B - Gas; AG - Acid and Gas; - - Negative.

Table 4: Confirmatory Test

S. No.	Pipe-borne Water	Well Water
1.	+	+
2.	+	+
3.	-	-
4.	-	+
5.	+	+
6.	+	-
7.	+	-
8.	+	+
9.	-	+
10.	-	-

KEY: + = Colonies were bottom like in appearance and surrounded by a greenish metallic sheen, flat with dark and black centers.

Table 5: Complete Test		
S. No.	Pipe-borne Water	Well Water
1	A	A
2	G	G
3	-	-
4	-	AG
5	AG	AG
6	A	-
7	G	-
8	G	G
9	-	G
10	-	-
KEY: A - Acid; B - Gas; AG - Acid and Gas; - - Negative.		

CONCLUSION

In conclusion, *Escherichia coli, Citrobacter* and *Klebsiella* were detected as the main water contaminants in Enugu East, Enugu State of Nigeria.

RECOMMENDATION

As a result of the above findings, It is therefore suggested that people living within Enugu East, Enugu State of Nigeria, should treat their water especially the drinking water before consumption. Treatments like boiling, distillation and filtration could remove some of these microbial contaminants in the water.

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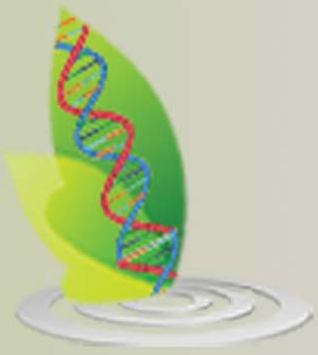

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