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Evaluation of indicator organism in water sources in two local government areas of Anambra State

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Abstract

Microbial quality of water using indicator organisms in water sources was investigated in two Local Government Areas in Anambra State, between the months of September and December, 2013. A total of 200 water samples were analyzed using Bacteriological Analytic method and membrane filtration method. The results showed that out of 200 different water samples examined organisms detected included, Escherichia coli 107 (26.75%).Streptococcus faecalis 112 (28.00%), Clostridium perfringes 45 (11.25%), Pscudomonas aeruginosa 19 (4.75%), Proteus mirabilis 46 (11.50%), Citrobacter freundii 70 (17.50%), Shigella Flexneri 1 (0.25%). This study portrays the diversity of indicator organisms in water sources in the study area. There is need therefore to put in place appropriate intervention strategies to control the spread of the indicator organisms from these sources to human population.

Keywords: Indicator organisms, Water sources, 2 Local Government Areas of AnambraStae

Introduction

Water is an essential part of human nutrition, both directly as drinking water or indirectly as constituent of food; in addition to various other applications in daily life. Water is not only essential for life, it also remains a most important vehicle of transmitting disease and infant mortality in many developing countries and even in technologically more advanced countries (Ford, 1999).

Good quality water is odorless, colourless, tasteless, and free from fecal pollution (Ezeugwunne*et al.*, 2009). However, it is estimated that about 1.2billion individuals world-wide do not have access to potable water. In many developing countries, availability of water has become a critical and urgent problem and it is a matter of great concern to families and communities that depend on non-public water supply system (Okonkoi*et al.*, 2008). Increase in human population has exerted an enormous pressure on the provision of safe drinking water in developing countries (Umeh*et al.*, 2005). To curb this health problem, bottled water was introduced but only individuals who have a good financial status can afford these products. Low income earners are left with no option but to consume sachet water, borehole water, wells, reservoirs, surface waters that are usually not free of microbial contaminants (Oladipo*et al.*, 2009). The potential ability of drinking water to transmit microbial pathogens to great number of people causing subsequent illness is well document in many countries at all levels of economic development (Dufour*et al.*, 2003). Water pollution has continued to create negative impacts on health and economic development in Nigeria (Ashbolt, 2004). High incidence of childhood diarrhoea, helminthiasis, trachoma, typhoid fever and the overall high mortality

rates are associated with poor environmental sanitation (Mengesha*et al.,* 2004). Therefore, this study evaluates four indicators of drinking water quality *(Escherichia coli, Clostridium perfringes, Total Coliforms, Streptococcus faecalis*) from drinking water source to determine the portability of the water.

The use of indicator organisms, in particular the Coliform group, as a means of accessing the potential presence of water-borne pathogens has been paramount to protecting public health (Hijnen*et al.,* 2000). These are based upon the principle of the detection of selected bacteria that are indicative of either contamination or deterioration of water quality through the use of simple bacteriological tests (Environmental Agency, 2002). Many pathogens are present in water only under specific conditions as a result of fecal matter from sewage discharges, leaking septic tanks, and run-off from animal feed lots bodies of water and, when present, occur in low numbers compared with other Micro-organisms. Whilst the presence of Coliform bacteria does not always indicate a public health threat, their detection is a useful indication that operations should be investigated (Edberg *et al.,* 2000).

Studies carried out on bacteriological quality of municipal borehole waters in Imo state, Nigeria revealed the presence of *Bacillus* (70%), *Pseudomonas* (80%), *Micrococcus* (50%), *E. coli* (40%) and *Proteus* (20%) in 320 water samples analyzed (Duru*et al.,* 2012).

As a result of the serious consequences and threats to health and economic development which water pollution poses, this work is therefore embarked upon to ascertain the microbial quality of drinking water with the following aim and objectives

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Aim

To evaluate the microbial water quality of various domestic water sources by the presence of indicator organism and *Salmonella species.*

Materials and Methods

Study area

This work was carried out in some selected communities in Idemili North and South LGA. Idemili North was represented by Oraukwu and Abatete while Idemili South by Nnobi, Alor and Nnokwa.

Data collection

Questionnaire was used to collect demographic data and other details. English language combined with local dialet was also used in administration of the questionnaire.

Methods of sample collection

A total of 220 water samples were collected, 50 samples were collected each from these water sources (well, borehole, reservoirs and surface water) and 20 controls (sterile water) between the months of September and December 2013.

Two hundred mls volume brown bottles in Triplicates were sterilized using Autoclave at 121°c for 15 minutes. Each bottle was labeled and numbered with the following information – Name of the sample site, time of sample collection, temperature of the water source.

Well water samples were collected into sterilized bottles tied with a strong twine to a piece of metal as the weight (EPA, 2012). The bottles were aseptically opened and lowered into the well. When filled, the bottles were gently raised to the surface and covered.

For collection of surface water samples, water was collected aseptically using hand, and grab method (EPA, 2012) and sample container positioned such that the mouth of the container is pointed against water current.

For borehole water samples, nozzles were properly cleaned and sterilized with cotton wool soaked in methanol. The water was turned on and allowed to run for two minutes after which, sterilized containers were carefully uncapped and held under the running tap.

For reservoir water samples, a piece of cotton wool soaked in methanol was ignited and used to heat-up the tap nozzle until it became unbearably hot to touch to avoid external contamination. The water was then allowed to run continuously for about one minute to cool the water after which the sample bottles were filled with water and covered carefully.

Storage and transport

The water samples were immediately stored in an ice box at low temperature and immediately transported to the laboratory. Samples were analyzed within 6 hours of collection (Cheesbrough, 2005).

Isolation of *Clostridum perfringes*

Procedure

Water sample of 50ml were poured into sterile bottle and the tubes were put in water bath maintained at 60^0C for 15 minutes to kill the vegetative spores. The water sample were allowed to cool and transferred into 75ml of cooked meat medium and incubated at 37^oc for 24hours.

The cooked meat medium with positive growth (turbidity) was filtered through the membrane filter of pore size 0.45µm.

Selective enrichment

Reinforced Clostridal medium was made differential for sulphite reducing Clostridia by the addition of 4% sodium sulphite and 7% ferric citrate.The membrane filter with Clostridium organism on it was placed on differential Reinforced Clostridal Medium and lncubated anaerobically using anaerobic jar at 44^0c for 24 hours. The plates were examined for the presence of Sulphite reducing *Clostridium*.

Total viable counts for *Clostridium perfringes* were done using the formula:

$$\text{Count/100ml} - \frac{\text{Number of colonies on membrane filter}}{\text{Volume of sample filtered}} \times \frac{100}{1}$$

(Microbiology of Drinking water 2004)

Purification

Suspected colonies were streaked onto blood agar plate for purification. The plates were incubated anaerobically at 37^0C for 24hrs. The plates were examined for zones of haemolysis.

Biochemical test for confirmation of *Clostridium perfringes*:

This was done according to the methods described in Health Protection Agency, 2004.

Buffered nitrate-motility test

This test is used to differentiate members of the Enterobacteriaceae that produce the enzyme nitrate reductase, from Gram negative bacteria that do not produce the enzyme.

Method

Immediately prior to use, the medium was heated in boiling water for 10-15 minutes to dissolve the agar and were allowed to cool rapidly to set. The isolated organisms were inoculated into the medium by stabbing on the media and incubated under anaerobic condition at 37^0C for 24 hrs.

After incubation, the medium were examined for growth along the line of the stab. Equal volumes of nitrate reagent A (Sulphanilic acid 0.83 and Acetic acid 100ml) and nitrate regent B (Cleve's acid 0.6g and 100ml acetic acid) were mixed immediately before use and presence of nitrate were tested by adding 0.5ml of these mixture to each of buffered nitrate motility medium. The formation of a red colour confirmed the presence of nitrate produced by the reduction of nitrate.

Small amounts of zinc dust were added to tubes that do not develop red colour and allowed to stand for 15minutes.
The test was considered negative upon development of a red colour meaning that no reduction of nitrate has taken place. The tests tube that did not develops red colour following addition of zinc dust mean that no nitrate remained and have been completely converted to nitrogen were recorded as positive.

Lactose-gelatine medium

This test is used to differentiate members of the Enterobacteriacaee that produce acid from lactose and gelatin liquefaction.

Method

Immediately prior to used, the medium was heated in boiling water for 10-15 minutes to dissolve the agar and were allowed to cool rapidly. The medium were incubated with the pure isolated organisms and incubated anaerobically at 37^0C for 24hrs. The tubes were examined for yellow colour indicating the production of acid. The tubes were then chilled for 2hours at 4^0C and examined for gelatin liquefaction. After solidification of the medium, the tubes were incubated at 37^0C for an additional 24hours after which the agar examined for gelatin liquefaction.
Clostridium perfringes produces black or grey to yellow brown colonies in differential reinforced *Clostridium* medium, is non motile, reduces nitrate to nitrite, produces acid and from lactose and liquefies gelatin within 37^0C ± 4 hours

Isolation of total and faecal coliform by membrane filtration method procedure

Water samples (100ml) were filtered aseptically through a Milipore Membrane Filter of pore size 0.45um. The membrane filter with the coliform organism on it was transfered and placed aseptically with the aid of blunt edge forcep on 2ml Chrom agar ECC liquid plate containing Cellulose acetate pad. The plates were incubated at 37^0c for 24hours. The plates were examined for appearance of purple and blue colonies on the Membrane Filter.

Total coliform counts were calculated using the formula below:

$$\text{Count/100ml} = \frac{\text{Number of colonies on membrane filter} \times 100}{\text{Volume of sample filtered} \quad 1}$$

Isolation of *Streptococcus faecals*

Streptococcus faecalis was isolated by Membrane Filtration

Water samples (100ml) were filtered aseptically through a Millipore membrane filter of pore size 0.45um. The Membrane Filter with the organism on it was transfer and placed aseptically with the aid of blunt edge of forcep on *Streptococcus faecalis* agar plate. The plates were incubated at 37^0c for 24hours. The plates were examined for presence of colonies pinkish appearance on the Membrane Filter.

Streptococcus faecalis counts were done using the formula

$$\text{Count/100ml} = \frac{\text{Number of colonies on membrane filter} \times 100}{\text{Volume of sample filtered} \quad 1}$$

Statistical analysis:

Statistical Analysis of results was done using Anova, T-test, Chi-square. Level of significant was set at 95% confidence limit and 0.05 levels respectively.

Statistical Package for Social Science (SPSS) Software Version 16 was used for evaluation.

Results

Table 1: Microorganisms detected from different water samples.

MICRORGANISM (CFU/100ml)	FREQUENCY	PREVALENCE (%)
Escherichia coli	107	26.75
Streptococcus faecalis	112	28.00
Clostridium perfringes	45	11.25
Pseudomonas aeruginosa	19	4.75
Proteus mirabilis	46	11.50
Citrobacterfreundii	70	17.50
Shighellaflexneri	1	0.25
Total	453	100.0

Table 2: Prevalence of total Indicator organism isolated from water bodies in Idemili North (Abatete and Oraukwu).

SOURCES	STREPTOCOCCUS FAECALIS (CFU/100ml)	FAECAL COLIFORM (CFU/100ml)	TOTAL COLIFORM (CFU/100ml)	CLOSTRIDIUM PERFRINGES (CFU/100ml)	TOTAL
ORAUKWU					
SURFACE WATER	*13(6.3)*	*13(6.25)*	*41(6.25)*	*35(6.24)*	102(51.0)
BOREHOLE	1(12.5)	2(6.9)	***9(6.2)***	***12(6.45)***	**24(12.0)**
ABATETE					
RESERVIOR	6(6.1)	*6(8.2)*	*21(6.4)*	*15(7.89)*	*42(21.0)*
BOREHOLE	*1(12.5)*	*0*	*8(6.25*	*0*	*9(4.5)*
RESERVIOR	*0*	*2(9.5)*	*7(43.7)*	*14(87.5)*	*23(11.5)*

Table 3: Prevalence of total Indicator organisms isolated from water bodies in Idemili South (Nnobi, Nnokwa and Alor).

SOURCES	STREPTOCOCCUS FAECALIS (CFU/100ml)	FAECAL COLIFORM (CFU/100ml)	TOTAL COLIFORM (CFU/100ml)	CLOSTRIDIUM PERFRINGES (CFU/100ml)	TOTAL
NNOBI					
SURFACE WATER	*19(5.7)*	*22(5.5)*	*59(6.3)*	*42(5.5)*	142(48.86)
BOREHOLE	2(10)	4(10)	*3(10)*	*6(10)*	14(4.62)
RESERVIOR	7(5.5)	*0*	*9(5.6)*	*2(5.6)*	*18(2.64)*
ALOR					
SURFACE WATER	*1(3.8)*	*1(4.0)*	*2(6.1)*	*1(4.8)*	*5(1.65)*
BOREHOLE	*1(5.9)*	*2(6.3)*	*9(5.5)*	*5(5.9)*	*17(5.61)*
RESERVIOR	*2(7.1)*	*4(5.8)*	*25(5.7)*	*13(5.8)*	*44(14.5)*
NNOKWA WELL	*24(2.6)*	*14(2.6)*	*22(2.6)*	*3(2.3)*	63(20.79)

Table 4: Condition of water sample sources.

VARIABLE SOURCES OF WATER	FREQUENCY	PREVALENCE (%)	P – VALUE
Borehole	379	49.34	0.000*
Other sources	101	64.35	
STORAGE MODE OF THE BOREHOLE			
Plastic Overhead tank	40	80.0	0.000*
Metal Overhead tank	10	20.0	
REGULAR WASHING OF TANKS			
Yes	32	68.0	0.000*
No	48	90.5	
HOW OFTEN DO YOU WASH TANK			
Every 1-3 weeks	6	30.0	0.000*
4-6 weeks	26		
6 weeks and above	48	92.5	
DEPTH OF THE BOREHOLE			
Less than 350ft	35	83.5	0.224**
Greater than 350ft	14	87.3	
SANITATION OBSERVATION WITHIN 10 METRES OF PIPELINE			
Yes	21	75.00	
No	59	81.9	0.002*
DEFECATION NEAR THE STREAM			
Yes	128 people	49.21	0.000*
No	352 people	53.69	

Key: * - significant; ** - not significant. **NB:** P-value =<0.05 significant, >0.05 not significant

Discussion

The presence of these organisms in some of the water source analysis is indicative of poor microbiological quality of the water samples.The World Health Organization (WHO, 2006) recommended not more than one *E. coli* and coliform colony per 100ml of water samples.

-This present study reveals high total Coliform count present in the entire water sample. This is indicative of pollution with human excreta. The presence of this indicator organism may suggest the possible presence of pathogens causing cholera, typhoid, gastroenteritis etc. this call for an initial treatment before consumption of water such waters are consumes. According to USA EPA standards water samples in which coliforms are detected should be considered unacceptable for drinking water as they are regarded as the principal indicators of water pollution. Only one body of water (borehole) in IdemiliNorth examined met the WHO recommendation for faecal coliform. Even then all other parameters did not meet accepted criteria and so cannot be good for consumption.

Twenty (20) percent of water sample in this present work contained *Clostridium perfringes.* Most *Clostridium perfringes* are non-pathogens, however, some strains are pathogenic as they have been implicated in endotoxin production (Crunickshank*et al.,* 2004). Their presence indicates faecal contamination.

Occurrence of *Pseudomonas* recorded in this present study can be justified by their wide spread distribution in aquatic and soil Ecosystem (Rogers *et al.,* 2005). Some species of *Pseudomonas* belong to the category of opportunistic pathogens which do not normally exist in animal host, but which can establish infections in individuals whose natural resistance has been reduced (Rogers *et al.,* 2005).

From this present work, it shows that water samples from wells had the highest prevalence rate of coliform when compared to water from other sources. This is not surprising because the individuals in this community engage in some unhygienic practices such as gardening without being conscious of personal hygiene. Most of them generally carry out a lot of domestic activities with dirty and unwashed hands especially after household chores like gardening, baby nursing and kitchen works.

The inhabitants in these communities engage in farming like poultry, piggery and goat rearing within the site of these wells. The surrounding flood water may contaminate water which find their way into these wells, as observed by Onyemenam (2013) who documented similar quantity of Enterobacteriaceae in an open well when compared with close well (borehole) in well water comparative study.

This study, as expected shows that there is high bacteria load in the surface river water sample. These findings are in agreement with those reported by Dougharie*t al.* (2007) in Tiete River in Brazil and Abednego *et al.* (2013) in Nairobi respectively. This study reveals that contamination of this surface water is by humans and other animal activities like bathing, farming and washing.

From this present study, it shows that boreholes can also be contaminated through flood water which forms after rainfall, depending on the depth of the boreholes. Deeper boreholes contain little or no micro organisms since they are usually removed by extensive filtration as water percolates through the soil (Uzoigwe and Agwa 2012) as shown by the Nnobi borehole constructed by the government with depth 350ft when compared to borehole constructed by individuals in Alor, Oraukwu and Abatete who have little or no knowledge about importance and role of depth in sanitation of water.

Contamination could also be through broken underground pipes under this condition, the surrounding flood water flows into the pipe through the cracks (Nwachukwu and Otokunafor, 2006; Orji et al. (2006) and several others have reported similar findings and recommended that household boreholes should be sited far away from soak-away pits and latrines.

Conclusion

The result of this study revealed that the water sources in these communities are highly polluted and is not safe for use by the communities for drinking or other domestic needs without prior treatment. There is need to monitor regularly and mitigate the effects of community behaviour on surface waters.

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