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MICROBIAL QUALITY ASSESSMENT OF WELL WATER IN KADUNA NORTH LOCAL GOVERNMENT

[1]Yabaya A. and Aliyu A.
Department of Microbiology
Kaduna State University, Kaduna, Nigeria
amosyabaya@gmail.com

ABSTRACT
The microbial quality of water in Kaduna north local government of Kaduna state was assessed, using multiple tube fermentation technique. A total of 20 well water samples within Kaduna north were analyzed for the presence of coliform bacteria. No single sample recorded the absence of coliform bacteria. Samples from location B and K had high most probable index with the range of 170-1600 coliforms per 100ml of the sample. Samples from location M and U had most probable index of between 27- 63 coliforms per 100ml of the sample. The isolates were identified to be *E. coli* and *Enterobacte*r species by biochemical characterization. These findings indicated that, the wells were contaminated.
Keywords: *Micrbial•Quality Assessment•Well Water•Kaduna North•Nigeria*

INTRODUCTION
The microbial examination of water is used worldwide to monitor and control the quality and safety of various types of water. As many potential pathogens could be associated with water; it is thus impractical to screen samples for all possible pathogens. Instead, various indicator organisms have been used as surrogate maker of risk. Most water borne diseases such as typhoid fever which is the most common known water born disease and cholera are related to faecal pollution of the water source (Hunter, 1997). Presence of coliforms (a collection of relatively harmless microorganisms that live in large numbers in the intestines of man and warm and cold-blooded animals) in water indicates its contamination with faecal matters, which is the greatest risk to human and can results to serious ill health (Cheesbrough, 2006). Recently however, public experts have recognized that certain protozoa, which cause diseases such as *Giardia* and *Cryptosporidium,* can be present in water even when total coliform test shows absence. But despite that, the total coliform test still remains the standard for determining the microbial quality of drinking water (EAI Analytical Labs). Being well as one of the major source of water in Kaduna north axis and its potential of becoming contaminated with faecal matters provide the need of assessing the quality and safety of the well waters in the axis.

MATERIALS AND METHOD
Sample Collection
A total of twenty samples from different wells in different locations within Kaduna north were randomly collected. The method adopted was that described by Cheesbrough, (2006). Sterile string was tied on a sterile 100ml capacity weighted bottle which its cap was aseptically removed and then lowered into the well to a depth of about 1m. The bottle was carefully raised out of the well when there were no bobbles rise to the surface and the cap was

carefully replaced. The bottle was then labeled and was taken immediately to the laboratory for analysis.

DETECTION OF COLIFORM ORGANISMS

The technique employed for the detection of the coliform organisms is the multiple tube fermentation (most probable number (MPN)) technique as described by Willey, *et al.* (2008).

PRESUMPTIVE TEST

Three sets of tubes each containing five tubes containing 10ml of Mackonkey broth, fitted cap and inverted Durham's tube were provided. The Mackonkey broth contained in the first set of the tubes is double strength while single strength in the other sets. 10ml of the water sample was added to each of the five tubes of the first set and labeled. 1ml and 0.1ml of the sample were added to each of the five tubes of the second and third sets respectively; and labeled accordingly. The tubes were loosely capped and incubated at 35-37^{o}C for 24 hours after which were examined for gas and acid productions. Positive tubes were identified by both gas production shown by collection of bobbles in the inverted Durham's tubes; and acid production shown by change in colour of Mackonkey broth from purple to yellow. Positive tubes were subjected to confirmatory test. The negative tubes were re-incubated at the same condition for total of 48 hours and re-examined for gas and acid productions.

CONFIRMATORY TEST

Positive tubes from the presumptive test ware paired with new tubes each containing similar content to its positive pair. Using sterile wire loop, the new pairs were inoculated with their corresponding positive pairs and inoculated for 24 hours at 35-37^{o}C. Positive tubes confirmed the presence of lactose fermenters in the water sample.

COMPLETED TEST

This test is to confirm the lactose fermenters were coliforms not Gram positive bacteria. Positive tubes from confirmatory test were inoculated on Levine's eiosin methylene blue (EMB) agar using streaking method and incubated for 24 hours at 35-37^{o}C . Coliforms' presence was confirmed by nucleated (dark center) colonies as methylene blue content of the medium inhibits the growth of Gram positive bacteria. The Most Probable Number (MPN) of coliform bacteria in 100ml of water was determined using MPN probability table.

IDENTIFICATION OF BACTERIA

Pure culture of bacterial isolates were obtained by sub-culturing colonies from positive completed test on nutrient agar (NA) and incubated for 24 hours at 35-37^{o}C after which discrete colonies were stored in NA slants for further characterization and identification.
The colonial morphology on growth medium and cellular morphology under a light microscope were examined.

GRAM'S STAINING

A smear of the test organism was prepared on a slide, heat fixed and covered with crystal violet stain for 30-60 seconds. It was rushed with clean water. The water was tipped off, covered with iodine for 30-60 seconds and rushed with water. It was then decolorizes with 95% alcohol and rushed with water immediately. It was covered with safranin (counter stain) for two minutes and washed with water. The back of the slide was wiped, dried on staining rack and observed under microscope. Gram positive organisms appeared purple while negative appeared red.

BIOCHEMICAL TESTS

In order to further identify the isolated organisms, the following biochemical tests were carried out using methods described by Cheesbrough (2006) and Oyeleke and Manga (2008).

INDOLE TEST

This test is based on identifying enterobacteria with the ability of producing enzyme tryptophanase. The test organism was inoculated in 3ml of peptone water and incubated at 35-37^{o}C for up to 48 hours. 0.5ml of KAVAC'S reagent was added and shook gently. Red colour in the surface layer was examined within 10 minutes, the presence of which indicated that the test organism produced an enzyme tryptophanase which broke down tryptophan contained in the peptone water to indole, pyruvic acid and ammonia. The compound *p*-dimethylamino-benzaldehyde in the KOVAC'S reagent then reacted with the indole and produced red compound, hence the organism indole positive.

METHYL RED – VOGES PROSKAUER (MR - VP) TEST

The methyl-red (MR) test is based on identifying mixed acid fermenting bacteria that yield a stable acid end product. The voges proskauer (VP) test is base on identifying bacteria capable of 2,3 butanediol fermentation following mixed acid fermentation. Sample was inoculated into 5ml of MR –VP broth and incubated for 48-37 hours at 35-37^{o}C. 1ml of the broth was transferred into a small serological test tube to which 2-3 drops of methyl red was added. Red colour on addition of the indicator indicated positive methyl red test. Five drops of 40% potassium hydroxide (KOH) was added to the remaining 4ml of the broth followed by 15 drops of 5% naphthol in ethanol. It was then shook, the cap was loosed and placed in a sloping position. Development of a red colour starting from the liquid – air interface within 1 hour indicated Voges proskauer positive test.

CITRATE UTILIZATION TEST

The test is based on the ability of an organism to utilize citrate as its only source of carbon. A slope of Simmon's citrate agar was produced. The sample was inoculated by streaking the slope with saline suspension of the test organism and stabbing the butt. It was then incubated for 48 hours at 35-37^{o}C. Bright blue colour in the medium indicated positive test while negative test was indicated by no change in colour.

LYSINE DECARBOXYLASE TEST

The test is based on the ability of the organism to ferment glucose and the production of an enzyme, lysine decarboxylase. The test organism was inoculated in lysine decarboxylase broth and layered with sterile paraffin oil. It was then incubated for 24-72 hours at 35-37^{o}C. The deepening or retention of purple colour indicated positive test.

HYDROGEN SULPHIDE (H_2S) PRODUCTION TEST

The test organism was inoculated on a slant of kligler iron agar (KIA) by streaking the slant's surface and stabbing the butt 2-3 times. H_2S production was indicated by blackening of the whole butt or streak path or ring of blackening at the slant butt junction after incubation for 24 hour at 35-37^{o}C.

RESULTS AND DICUSSIONS

The result obtained as shown in the table revealed that sample B3 recorded the highest coliforms count of 1600 per 100ml of the sample. Also other samples from same location recorded high counts. This is an indication of constant contamination of the wells in that location with faecal materials, probably due to improper sewage disposal, proximity of the wells to septic tanks or solid waste management facility such as landfill and dumping site; as most of private individuals do not consider distance of possible source(s) of contamination when constructing wells. Dug wells should at least be 30.5m a way from sewage disposal system and 61.0m away from solid waste management facility; and drilled wells should at least be 15.2m away from sewage disposal system and 61.0m away from solid waste management facility (NSE., 2009). Samples K1-K5 also recorded high coliform count ranged between 170-280 per 100ml of the water sample. Sample M1 on the other hand had the lowest coliform count of 26. Other samples from the same location and those from location U showed relatively low coliforms count compared to those in location B and K. Though water for drinking most possesses 0 MPN of coliforms per 100ml (WHO, 2006). The isolates were identified to be *E. coli* and *Enterobacte*r species Coliforms count of 26 per 100ml which happened to be lowest count is of serious public health concern.

CONCLUSION AND RECOMMENDATION

From the available result, it can be concluded that most well waters within Kaduna north did not satisfy coliforms standard for drinking water. It is therefore recommendable that wells should be well constructed. The distance between wells and possible source of contamination should be considered when locating site for well construction. Individuals should be examining their wells periodically to assess the quality and safety or otherwise of the water. Government regulatory agencies such as NAFDAC and SON should extend their periodic inspection to private water sources and if need be, enlighten the residence on control and preventive measures of contaminations.

Table: MPN index of coliform organisms per 100ml of sample

SampleCode	10ml	1.0ml	0.1ml	MPN/100ml
K1	5	4	3	280
K2	5	5	1	170
K3	5	5	0	240
K4	5	3	3	180
K5	5	4	2	220
B1	5	5	1	350
B2	5	5	3	920
B3	5	5	5	1600
B4	5	5	2	540
B5	5	5	3	920
M1	4	2	1	26
M2	4	3	0	27
M3	5	0	1	31
M4	4	3	1	33
M5	5	1	0	33
U1	5	0	2	43
U2	5	1	2	63
U3	5	1	1	46
U4	5	3	1	110
U5	5	3	2	140

Key: MPN= Most Probable Number.

REFERENCES

Brown, E.A. (2007). Benson's Microbiological application. 10th edition. McGraw Hill Companies Inc. New York. Pp: 387-395

Cheesbrough, M. (2006). District Laboratory practice in tropical countries part 2 Cambridge low price edition Pp: 38-39, 62-70 & 143-157

EIA Analytical Labs. Coliform bacteria in drinking water. Doc. Keene center.

Hunter, P.R. (1997) water-born disease: epidemiology and ecology. Chichester: wiley

Nova Scotia Environment. (2009). The drop on water, coliform bacteria. (www.gov.ns.ca/nsc/water).

Oyeleke, S. B. and Manga, B. S. (2008). Essentials of Laboratory practical in Microbiology. Tobest pub, Minna. Pp: 36 – 67

Willey, J.M., Sherwood, L.M. and Woolverton, C.J. (2008). Prescott, Herley and Klein's Microbiology. 7th edition. McGraw Hill Companies Inc. New York. Pp: 1050-1060

World Health Organization. (2006). Guidelines for drinking water quality. 1st addendum-3rd Edition . Vol: 1. Recommendations. Electronic version.

Appendix: List of sampling locations B- Badarawa, K- Kawo,M- Malali, U- Unguwan dosa