Isolation and Identification of Coliform Bacteria from Selected Well Water in Lapai Metropolis, Niger State, Nigeria.

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Abstract: The study was carried out to isolate and identify Coliform bacteria from well water situated in Lapai metropolis. Fifteen (15)samples of well water, used for domestic purposes were collected from different well water points within Lapai metropolis. Most Probable Number was used for enumeration of Coliform, Cultured on Nutrient agar and Eosin methylene blue (EMB) Agar. The isolates were subjected to series of biochemical tests for possible identification. The physicochemical parameters analyzed were pH, Temperature, and Turbidity. The MPN coliform index per 100ml of the water samples ranged from 0-1100+. The Bacteria isolated from well water samples included, *Escherichia coli* (26.7 %,) *Citrobacter* sp (13.3%), *Shigella* sp (13.3%), *Salmonella* sp(6.7%), *Klebsiella* sp (20.0%), *Serratia* sp (13.3%), *and Enterobacter* sp(6.7%). The percentage occurrence of the isolates indicated that *E. coli* was predominant with 26.7%, the least occurrence of the isolates were *Salmonella* sp, and *Enterobacter* sp with 6.7% each. The highest temperature recorded for the samples was 25.8°C, and the least was 20.7°C. The pH of the water samples ranged from 6.48-8.15. The turbidity values of the well water samples ranged from 0-4 Nephelometric Turbidity Units (NTU). From the results obtained, most of the water samples analyzed met World Health Organization standard of 0 MPN index/100ml. However, the few samples observed to harbour contaminations calls for treatment before consumption.
Key words: Isolation, Coliform bacteria, Well water

Introduction

Water is a colourless, odourless and tasteless compound, with chemical formulaH_2O, which is abundant in nature and is essential to life (Abera *et al.*, 2011). It is part of the physiological process of nutrition and waste removal from cells of all living things. It is one of the controlling factors for biodiversity and the distribution of Earths varied ecosystems, communities of animals, plant and bacteria and their physical and chemical interrelation (Chan *et al.*, 2007). In anabolism, water is removed from molecules (through energy requiring enzymatic chemical reactions) in order to grow larger molecules (e.g. starch, triglycerides and proteins for storage of fuels and information). In catabolism, water is used to break bonds in order to generate smaller molecules (e.g. glucose, fatty acids and amino acids to be used for fuels for energy use or other purposes). Without water, these particular metabolic processes cannot exist (Olayemi, 2007).

Water is said to be contaminated when harmful microbial or chemical agents are present in it, though it may have seemingly pleasing taste, odour and appearance. Polluted water is one with unacceptable appearance, taste and odour resulting from the introduction of domestic, industrial or agricultural waste causing an alteration in its natural state. Pollution may be in form of solid, liquid or gas and its sources could be physical, chemical or biological. The importance of biological pollutants cannot be undermine, as far as individual and public health is concerned (Amira and Yassir, 2011).

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Potable water is water which is fit for consumption by humans and other animals. It is also called drinking water, in reference to its intended use. Water may be naturally potable, as is the case with pristine springs, or it may need to be treated in order to be safe. In either instance, the safety of water is assessed with tests which look for potentially harmful contaminants. Potable water is water that is free of all objectionable material, including pathogens, tastes, odour, colour minerals, toxins, radioactive material, organisms, oils, gases e.t.c. it can contain high concentrations of some minerals (e.g. calcium and magnesium) and gases like carbon dioxide. Potable water has been described as water of sufficiently high quality that can be consumed or used without risk of immediate or long term harm. Therefore, portable water is free from injurious substances, pleasant to taste and satisfactory for drinking. To be safe, human drinking water may be free from organisms capable of causing diseases. Water for drinking should have a reasonable temperature and if treated with a disinfectant ch as chlorine, should have minimum level of chlorine that is harmless to the consumers but able to achieve its goal (Chan *et al.*, 2007).

Well water is a common source of drinking water during dry season in cities and villages where social amenities like pipe born water is still a dream. Well water has been sampled to contain little number of microorganisms owning to the filtering effect of natural percolation of rocks. Water and health are intricately linked. There are basically two types of links which facilitates elucidation of cause effects relationship between water and impacts on health: One is water conveyance medium of pathogens, and the other is water as providing the habitats for vectors and intermediate hosts of pathogens (Olayemi, 2007). Water related disease may be divided into those caused by pathogenic organisms and

those caused by some chemical substance in water. The first group may be called water-related infections and may include some of the greatest cause of diseases and death in developing countries. The second group includes diseases such as flourosis (linked to high fluoride levels in drinking water) and infantile methonoglobinemia (related to high nitrate level) in drinking water.

In Nigeria, water related diseases caused by pathogen can be further classified into water-borne disease, water based diseases and those transmitted by water-related insects or animal vectors (Olayemi, 2007). Microbiological contamination of water has long been a concern to the public. From 1920s – 1960s, the *Bacillus* sp which causes typhoid fever was considered a major problem in the water supply. Once it was eradicated, new microbes were present to take its place. In parts of the US, concern kept increasing due to outbreaks of coliform bacteria Giardiasis, Cryptosporidiosis, and Hepatitis A. (APHA, 2005). Water receives its microbial content from air, soil, sewage, organic waste and dead plant or animal remains. This clearly shows that, at times, almost any kind of organisms may be found in water. Most of the bacteria find the condition unfavorable and soon die, while those that survive are potentially transmissible to man through his use of the water for domestic purposes such as drinking, cooking and washing. The realization that water could serve as a major vehicle for disease transmission became wide spread only after the birth of the science of bacteriology in the year 1870 (Kravitz *et al.*,1999).

The fact that coliforms are commensals of the intestine, this does not rule them out of been pathogenic in other parts of the body. (Haruna *et al.*, 2005). The pathogens frequently transmitted by contaminated water are the causative agent of typhoid and paratyphoid fever, bacillary dysentery, unrinogenital infections and disease such as cholera. They are disseminated by the faeces of patients or healthy carriers. The coliforms are generally present in large numbers in human excrement and can be as small as 2 in 100ml of water. They are therefore, the most sensitive indicators at to demonstrate faecal contamination of water. The presence of coliform bacteria in water samples indicate that intestinal pathogens may likely be present, although in a much fewer number. Thus water is considered free from pollution when it contains less than one(1) per 100ml of water (WHO, 2006). In 2006, the WHO safety standard for potable water stipulate that the presence of faecal coliform should be less than 10 per 100ml of untreated water and less than one (1) coliform per 100ml of treated water. Members of colifoms organism include those which are classified in the genera *Escherichia, Citrobacter, Hafnia, Enterobacter* and *Klebsiella* (WHO, 2006).

Other inhabitants of the intestine that may be used as indicator of faecal contamination are faecal *Streptococci* and *Pseudomonas aeruginosa, Staphylococcus aureus, Micrococcus* sp and *Bacillus* sp. These organisms are present in most water that is consumed and they cause specific diseases (WHO, 2006).Other inhabitants of the intestine that may be used as indicator of faecal contamination are faecal *Streptococci* and *Clostridium perfringes* which are strictly anaerobic. Others include *Pseudomonas aeruginosa, Staphylococcus aureus, Micrococcus* sp. *Bacillus* sp. Coliform bacteria will not likely cause illness. However, because coliform bacteria are most commonly associated with sewage or surface waters, the presence of coliform bacteria in drinking water indicates that other disease causing organisms (Pathogens) may be present in the water system (Mengesha *et al.*, 2004). There are three different groups of coliform bacterial; each with a different level of risk such as total coliform, faecal coliform, *E coli.*

Materials and Methods

Sample Collection

Fifteen (15) samples of well water were collected from Lapai in already sterilized 200mls conical flasks. The samples were taken from four (4) different locations which were Badeggi, TankoIllimi, Soje, and Emir's palace in Lapai. Four (4) well water samples were taken from three (3) locations each and three (3) samples taken from one location and kept in the refrigerator to preserve them. The mouth of the sample containers were sterilized carefully with spirit lamp before putting inside the well, to allow for the collection of the sample into the sterilized bottles of 200mls. The sampling bottle was thereafter labeled with the desired code, and kept in the refrigerator to preserve them prior to usage.

Determination of the Most Probable Number (MPN).

The Most Probable Number method (MPN) was used for the enumeration of coliform isolates

Exactly (10ml) of double strength nutrient broth was dispensed into five(5) MacCartney bottles for each sample, and 0.1ml and 1ml of single strength nutrient broth was dispensed into 10 MacCartney bottles for each sample to be analyzed. Durham tubes were inserted vertically, and each bottle was covered and well labeled for detection of gas. The MacCartney bottles were then autoclaved at 121^{0}c for 15mins. After the autoclaving, the bottles which contained Nutrient broth were allowed to cool. Exactly (10ml) of the water sample was dispensed using a sterile 10ml syringe into the first five double strength broth, 1ml of the water sample was measured into five MacCartney bottles for single strength broth, and 0.1ml was introduced into the last five MacCartney bottles with single strength broth. The MacCartney bottles were incubated. Positive bottles show production of gas inside the Durham's tubes, colour change and turbidity was observed after 24hrs at 37^{0}C of incubation.

Presumptive Test

After the incubation of MacCartney bottles, the positive ones were selected. This was then cultured into Nutrient agar and EMB (Eosin Methylene Blue) agar using pour-plate method. Exactly 0.1ml of the liquid was inoculated into the plates and incubated for 24hrs at 37^{0}C.

Confirmatory Test

The Positive plates from presumptive test were sub-cultured onto Nutrient Agar using streak plate method and incubated at 37^{0}c for 24hours. Discrete colonies were observed.

Compleled Test

Nutrient Agar was prepared according to the manufacturer's specification. It was poured into slant bottles and autoclaved at 121^{0}c for 15 minutes and was allowed to gel in a slant position. The pure isolate sub-cultured from Nutrient Agar plates were inoculated on the slant bottles for preservation and for further biochemical tests. These biochemical tests included Gram-staining, Oxidase, Urease, Motility, Voges Proskauer, Indole, Citrate utilization, Methyl Red, Sugar fermentation, Etc.

Isolation of Bacteria

The pure isolates sub-cultured from Nutrient Agar plate were inoculated on the slant bottles for preservation and for further biochemical tests. This was done by using sterile wire loop to pick a single colony, inoculated into the slant bottle and incubated at 37^{0}c for 24hours.

Biochemical Characterization and Identification of Isolates

Characterization and identification of bacteria was done based on Gram – staining, Colony, morphology and Biochemical tests, These biochemical tests carried out includes, Urease test, Citrate utilization, Indole test, Methyl Red, Voges Proskauer, Motility, Sugar fermentation, (Oyeleke and Manga 2008; Cheesbrough, 2006). Bacterial isolates were identified by comparing their characteristics with those of known Tests, using Cowan and steel manual for identification of biochemical tests.

Physico-chemical Analysis

The control water sample (CWS) was the first sample to be analyzed. Physical analysis was first carried out. A little quantity of the sample (CWS) was poured into the beaker and the temperature was observed using conductivity meter, the result was recorded. pH meter was calibrated and the water sample was poured inside the beaker after a minute, thereafter, the pH meter was inserted into the water sample in the beaker, result was displayed on the pH meter and was recorded. The turbidity of the water sample was observed by placing some quantity of the sample in a space in the turbidity machine and the result displayed on the machine was recorded.

Results

Fifteen (15) water samples made of four (4) each in Badeggi, Tankoilimi, and Soje, and three (3) samples from Emirs Palace from Lapai metropolis were subjected to bacteriological analysis, using the Most Probable Number techniques (MPN). The result are recorded in the table, double strength was made of 10ml, single strength was made of 1ml and 0.1ml, the values of double and single strength all ranges from 0-3. The MPN/100ML range from 2-1100+ (Table 1)

Table 1:Presumptive Coliform Count in MPN

S/No	Sample Code	Double Strength 10ml	Single Strength 1ml	Single Strength 0.1ml	MPN/100ml
1.	BDG 1	3	3	3	1100+
2.	BDG 2	2	2	0	9
3	BDG 3	1	1	0	4
4	BDG 4	3	2	0	14
5	TKM1	3	0	1	11
6	TKM2	3	2	3	1100
7	TKM3	3	2	0	14
8	TKM4	3	3	3	1100+
9	SJ1	3	1	0	11
10	SJ2	1	0	0	2
11	SJ3	2	2	0	9
12	SJ4	3	3	3	1100+
13	EMP1	0	0	0	0
14	EMP2	0	1	0	2
15	EMP3	3	2	0	14

KEY:
BDG = Badeggi TKM = Tankollimi SJ = Soje EMP = Emirs Palace

The isolates ware subjected to different biochemical tests and the bacteria identify includes *Escherichia coli* (26.7 %,) *Citrobactersp* (13.3%), *Shigella sp* (13.3%), *Salmonella sp* (6.7%), *Klebsiella sp* (20.0%), *Serratia sp* (13.3%), *and Enterobacter sp* (6.7%).(Table 3). Among the isolated bacteria, *E coli* have the highest percentage while *Enterobacter sp* has the least percentage.

Table 2: Percentage Occurrence of the Bacteria in Well water.

S/No	Bacteria	Number	Percentage Occurrence (%)
1	*Escherichia coli*	4	26.7
2	*Citrobacter* sp	2	13.3
3	*Shigella* sp	2	13.3
4	*Salmonella* sp	1	6.7
5	*Klebsiella* sp	3	20.0
6	*Seratia* sp	2	13.3
7	*Enterobacter* sp	1	6.7
TOTAL		**15**	**100**

Physico- chemical analysis of the water samples was carried out on the fifteen (15) water samples. pH range from 6.48- 8.15, Temperature range from 20.70 °C- 25.80 °C, and Turbidity range from 0- 4 NTU.

Table 3: Physicochemical Parameters of the Water Sample

S/N	Sample Code	pH	Temperature (^{0}c)	Turbidity (NTU)
1	BDG1	8.15	20.7	3
2	BDG2	7.49	21.6	1
3	BDG3	6.67	21.5	0
4	BDG4	6.95	21.6	2
5	TKM1	6.88	21.4	3
6	TKM2	6.83	24.9	3
7	TKM3	8.95	24.9	4
8	TKM4	6.48	24.7	2
9	SJ1	6.55	25.0	3
10	SJ2	6.74	25.0	4
11	SJ3	7.50	21.6	0
12	SJ4	8.62	24.7	2
13	EMP1	6.92	23.55	3
14	EMP2	6.78	25.8	1
15	EMP3	7.45	21.49	4

KEY:
BDG = Badeggi TKM = Tankollimi SJ = Soje EMP = Emirs Palace NTU = Nephelometric Turbidity Units

Discussion

Well water from Lapai metropolis was subjected to bacteriological analysis using the Most Probable Number (MPN) technique. The result showed that only a sample in Emirs Palace of all the four locations investigated recorded count of 0 (MPN) index/100ml. This could be attributed to the generally clean environment of the area and the location of the well water from pit latrines and dirty environment. This is in conformity with the findings of Gerald *et al.* (1992) that says boreholes and well water that are properly located produce water of very good quality. The microbial load obtained from the well water samples apart from the sample from Emirs palace in this study indicated that there is danger in the usage of the water. This also agrees with Michael *et al.*(1993) who reported that the presence of coliforms in water is regarded as a warning signal: the water is subject to potentially dangerous pollution.

Seven different bacteria were isolated from the water samples. The bacteria were identified as *Escherichia coli*, *Citrobacter* sp, *Shigella* sp, *Klebsiella* sp, *Serratia* sp, *Enterobacter* sp, *Salmonella* sp. Thisis in conformity with the findings of Payment (1993) that reported bacteriological analysis of underground water samples to contain different species of bacteria that includes, *Enterobacter* sp, *Klebsiella* sp, *Citrobacter* sp, *Micrococcus* sp, *Proteus* sp, *Bacillus* sp and *Seratia* sp. *Escherichia coli* was the most frequent occurring isolate encountered in this study. This is similar to the findings of Oyedeji *et al.*(2002) who reported heavy bacteria load in water from some well water in Ilorin. The preponderance of this bacterium may be connected to poor refuse disposal close to some of the well water, most sample were obtained during the raining season

where the percolation of different contaminants may have occurred.

Escherichia coli are of considerable value as an indicator organism in bacteriological examination of water, because of its occurrence in faeces. *Escherichia* is aerobic and facultative anaerobic, its optimum temperature for cultivation is 37^{0}c but growth occurs at range of 40 -44^{0}C. (Ryan *et al.*, 2004).The presence of *E. coli* in a drinking water sample always indicates recent fecal contamination – meaning that there is a greater risk than other pathogens that are present. *Citrobacter* spoccured twice in the well water samples and has 26.7% while *Salmonella sp* occuredonce with 6.7%. This is in agreement with the findings of Oyedeji *et al.* (2009) who reported that *Salmonella* spoccured twice with 6.7% and *Citrobacter* sp occured 4 times with 13. 3% in underground water samples.

The pH of the water samples ranged from 20.7 - 25.8^{0}C and turbidity ranged from 0 - 4. This is in conformity with the findings of Medera *et al.* (1999) who reported pH of well water samples to range from 20.8 - 30.0^{0}C and the turbidity ranged from 0.4 - 0.8.

Conclusion

From the results obtained, most of the water samples analyzed met the World Health Organization standard of 0 MPN index/ 100ml. However, the few samples observed to harbor contaminations necessitate treatment before consumption. The consumption of untreated water can have serious health effects on humans. Providing adequate and safe drinking water aids in the safe guarding of the health and wellbeing of humans, and therefore of high priority all over the world. The ultimate safety of drinking water for municipal consumption depends on the protection of water sources, construction and maintenance of a very reliable and effective treatment plant and distribution system.

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