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Incidence of Faecal Coliform in Well Water Obtained from Sabon-Gari Area, Osogbo

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Abstract

Well water samples obtained from Sabon gari area of Osogbo, Osun state were analysed for the bacteriological qualities, physico-chemical and organoleptic properties. The spread plate technique was adopted using MacConkey, mannitol salt and thiosulphate citrate bile salt sucrose (TCBS) agars. The total coliform, faecal coliform, and staphylococcus counts on MacConkey and mannitol salt agar were 7.3 x 10^6, 3.9 x 10^6 and 1.1 x 10^6 cfu /mL respectively but there was no growth on TCBS agar. *Escherichia coli* (27%), *Klebsiella pnuemoniae* (19%), *Shigella dysenteriae* (25%), *Salmonella typhi* (16%) and *Staphylococcus species* (13%) were identified as the major bacteria isolated from the well water. The pH value ranged from 6.25 to 7.93 while the temperature ranged between 26.1 and 28.9^{0}C respectively. Microbiological quality is the most important aspect of drinking water in relation to waterborne diseases. Thus, detection of bacterial indicators in drinking water means the presence of pathogenic organisms related waterborne diseases.

Keywords: Well water; coliform; incidence rate; pH; temperature; *Escherichia coli*

Introduction

Water covers 70.9% of the earth's surface and is vital for all known forms of life . Access to safe drinking water has improved over the last decades in almost every part of the world, but approximately one billion people still lack access to safe water and over 2.5 billion lack access to adequate sanitation (CEH, 2005). Water plays an important role in the world economy, as it functions as a solvent for a wide variety of chemical substances and facilitates industrial cooling and transportation. Well water is susceptible to contamination from a variety of sources, including septic tanks, pesticides, and household chemicals. However, shallow and permeable water table aquifers are most susceptible to contamination (Borchardt *et al.*, 2003).

Contaminants such as bacteria, viruses, heavy metals, nitrates and salt have polluted water supplies as a result of inadequate treatment and disposal of waste from humans and livestock, industrial discharges, and over-use of limited water resources (Wright *et al.*, 2004). The geological nature of the soil determines the chemical composition of the groundwater. The water potential to harbor microbial pathogens and

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cause illness is reported for both developed and developing countries (Wright *et al.*, 2004). Introduction of pollutants into the natural water occur directly through point source (septic tanks, disposal sites etc.) near the ground water or indirectly through non-point source when already polluted water in the area enters into the freshwater body by lateral or side movement (Borchardt *et al.,* 2003). Water pollution results in transmission of infectious diseases. The implications of waterborne bacteria and virus infection include polio, hepatitis, cholera, diarrhea, typhoid etc (Nassinyama *et al.,* 2000).

Faecal material is an indirect source of decomposed organic matter (DOM) as the solids are solubilized during microbial degradation (Lacroix and Gregoire, 2002). Recognized bacterial indicators for assessing water quality are bacteria of the *Enterobacteriaceae* family defined as total coliform bacteria and the fecal coliform bacteria. The coliform bacteria are gram-negative rods, with the faecal coliform bacteria usually, but not always, found in the faeces of warm blooded animals: The presence of faecal coliform bacteria in water is indicative of contamination by faecal material and considered indicative of health risk. Significance of coliform group density is established as an indication of the degree of pollution and the sanitary quality of water.

In Nigeria, increasing population and infrastructural breakdown have made municipal pipe borne water to be inadequate in quantity and quality but, less than 30% Nigerians have access to safe drinking water due to these inadequacies and most of the populations have to resort to drinking water from wells and streams especially in the rural and suburban communities (Oyedeji *et al.,* 2011). These water sources are largely untreated and might harbour waterborne and vector-borne diseases such as cholera, typhoid fever, diarrhoea, hepatitis and guineaworm (Rahman *et al.*, 2001). The aim of the study was to determine the physical, organoleptic and incidence of faecal coliform in well water samples.

Materials and Methods

Collection of samples

A total of thirty- three (33) well water samples were collected from different locations at Sabon-gari area of Osogbo, Osun state within December, 2010 and March, 2011. The water samples (1 L) were aseptically collected (triplicate) into a sterile universal bottle and transported to the laboratory for microbiological analysis.

Assessment of Physical and Organo-leptic properties of well water sample

Physical parameters such as pH and temperature were measured directly on-site by conventional methods using portable pH meter (Jenway, model 3270) and thermometer. Organoleptic properties such as colour, taste and odour were observed and recorded respectively.

Isolation and Enumeration of faecal coliforms

Each well water sample was serially diluted with sterile peptone water to obtain dilutions of 10^{-2}, 10^{-3} and 10^{-4} respectively. Aliquots of 0.1 mL of the 10^{-4} dilution was aseptically inoculated onto the solidified agar media using a spread plate method. MacConkey agar plates were incubated at 37°C and 44.5°C for 48hours for isolation of coliforms and faecal coliforms respectively. For isolation and enumeration of *Staphylococcus species*, *Salmonella species*, *Shigella species* and *Vibrio species*; Mannitol salt agar, Salmonella-shigella agar and Thiosulphate citrate bile salt-sucrose agar plates were inoculated and incubated at 37°C for 48hours respectively.

Purification and Maintenance of isolates

The morphological and colonial characteristics of the isolates were observed and recorded. Each distinct bacterium colony was sub-cultured on nutrient agar plates to obtain pure colonies. The purified bacteria isolates were maintained on nutrient agar slants and stored in the refrigerator (4°C). The presumptive faecal coliform colonies were sub-cultured on Eosin methylene blue agar to differentiate between *Escherichia coli* and *Klebsiella spp.*

Morphological and Biochemical characterization of isolates

The isolates were characterized to the species level on the basis of their morphological and biochemical characteristics. Distinctive morphological properties of each pure culture such as colony form (appearance, colour, shape and size of the colony on the agar medium), elevation of colony, microscopic morphology and biochemical test such as catalase, coagulase, motility, oxidase, citrate and carbohydrate fermentation test were done based on the method of Borchardt *et al.* (2004).

Results

A total of 84 bacterial isolates were selected based on their morphological and biochemical characteristics. These include *Escherichia coli* (23 strains), *Shigella dysenteriae* (21 strains), *Staphylococcus aureus* (11 strains) *Klebsiella pneumoniae* (16 strains) and *Salmonella typhi* (13 strains) respectively. *Vibrio species* were absent in all samples analyzed. The incidence rate of the isolates obtained is shown in figure 1. High faecal indicator bacteria concentrations were obtained from all water samples. The colonial morphology, microscopic morphology, biochemical test and the microbial load are shown in table 1 and figure 2 respectively.

The physical water quality parameters that were considered in the monitoring program were temperature, pH, taste, odour and colour (table 2). Temperature is a fundamental factor for water quality, exerting a great influence over the aquatic system. pH is a known standard measure of acidity and alkalinity. The temperature of the water sample ranged between 26.1 and 28.9^{0}C while the pH value ranged from 6.25 to 7.93 respectively.

Discussion

Well-water at Sabon-gari area of Osogbo, Osun state is utilized for domestic purposes and drinking-water should ideally have no visible colour. Coloration or cloudiness of well water is usually due to the presence of coloured organic matter (primarily humic and fulvic acids) associated with the humus fraction of soil (Borchardt *et al.,* 2004). Colour is also strongly influenced by the presence of iron and other metals, either as natural impurities or as corrosion products. It may also result from the contamination of the water source with industrial effluents and may be the first indication of a hazardous situation. Odour can originate from natural inorganic and organic chemical contaminants and biological sources or processes (e.g. aquatic microorganisms), from contamination by synthetic chemicals, from corrosion or as a result of water treatment (e.g., chlorination). Although 66.67% of samples analyzed had pH within an acceptable range (6.5 to 8.5) while 33.33% were acidic (pH 6.25-6.49) and thus less desirable (Table 2) because the pH range of drinking water according to WHO recommendation is 6.5 to 8.5 (WHO, 2004). Water is constantly in contact with the ground in which it stagnates or circulates, so equilibrium develops between the composition of the soil and that of the water: i.e. water that circulates in a sandy or granitic substratum is acidic and has a few minerals while water that circulates in limestone contains bicarbonates alkalinity.

Verification of the microbial quality of drinking-water includes testing for *Escherichia coli* as an indicator of faecal pollution. *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Shigella dysenteriae* and *Salmonella typhi* are the major bacterial species encountered in most of the well water samples analyzed (Table 1 and fig.1) and presence of such organisms agrees with the report of Idowu *et al.* (2011). The mean total coliform, faecal coliform and staphylococcus counts of 7.3×10^6 cfu/mL, 3.9×10^6 cfu/mL and 1.1×10^6 cfu/mL (Figure 2) respectively are higher than the recommended World Health Organization (WHO) limit which is 5% for total coliform and free for faecal coliform (WHO, 2007). Furthermore, the high incidence rate of gram-negative rods especially *E. coli, Shigella dysenteriae* and presence of *Staphylococcus aureus* agrees with the report of Barrell *et al.*, (2000). Summarily, the presence of faecal coliforms in well water sample is an indication of faecal contamination. *E. coli* provides conclusive evidence of recent faecal pollution and should not

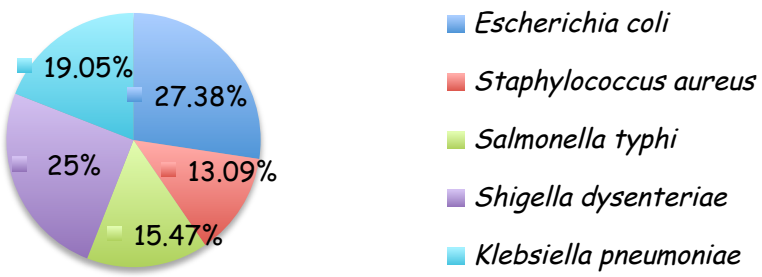

Figure 1: Incidence rate of bacterial isolates obtained from well water samples

Table 1: Morphology and biochemical characteristics of isolates obtained from well water samples

CM	GR	OX	LA	D X	SU	CA	CO	CT	UR	MT	ISOLATE
Pinkish smooth colony on macConkey agar	Gram negative rod	-	AG	AG	A	ND	ND	-	-	+	*Escherichia coli*
Pinkish colony on macConkey agar	Gram negative rod	-	AG	AG	AG	ND	ND	+	+	-	*Klebsiella pneumoniae*
Pale colony without black spot on salmonella-shigella agar	Gram negative rod	-	-	A	A	ND	ND	-	-	-	*Shigella dysenteriae*
Pale colony with black spot on salmonella-shigella agar	Gram negative rod	-	-	-	G	ND	ND	-	-	+	*Salmonella typhi*
Yellowish colony on Mannitol salt agar.	Gram positive cocci	ND	A		A	+	+	-	-	ND	*Staphylococcus aureus*

Key: CM = Colonial morphology AG = Acid and gas produced
GR = Gram reaction G = Gas produced
DX = Dextrose - = No reaction
SU = Sucrose + = Reaction occurred
LA = Lactose UR = Urease test
OX = Oxidase test ND = Not determined
CA = Catalase test
CO = Coagulase test
CT =Citrate test

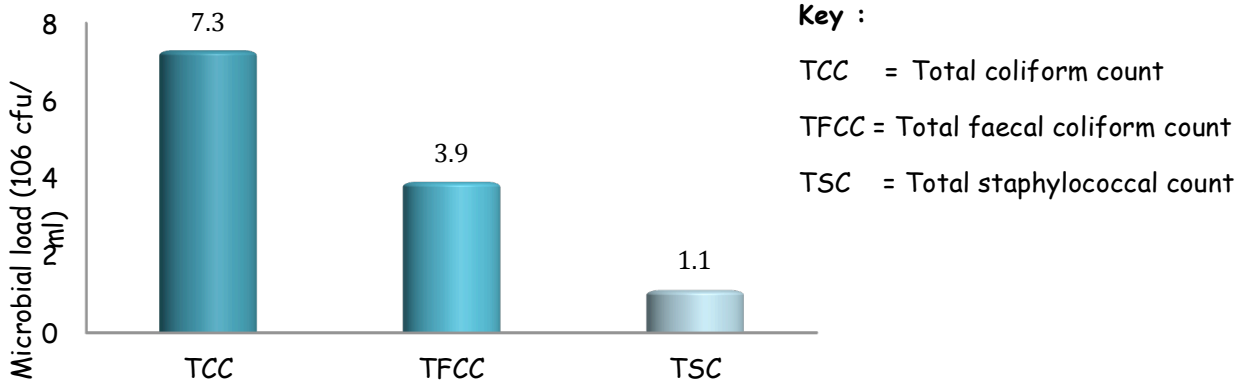

Figure 2: Enumeration of microorganisms present in well water

Table 2 : Physico-chemical properties of well-water samples

Sample code	Colour	Odour	Temperature (mean±SD)	pH (mean±SD)
WL 01	Colourless	Odourless	26.3± 0.04	6.81± 0.01
WL 02	Colourless	Odourless	27.8± 0.17	7.93± 0.06
WL 03	Cloudy	Metallic	28.5± 0.05	6.27± 0.12
WL 04	Colourless	Odourless	26.5± 0.11	6.85± 0.14
WL 05	Cloudy	Metallic	28.9± 0.01	6.29± 0.17
WL 06	Colourless	Odourless	28.1± 0.18	7.86± 0.03
WL 07	Colourless	Odourless	27.5± 0.09	6.92± 0.01
WL 08	Cloudy	Metallic	28.8± 0.12	6.39± 0.15
WL 09	Colourless	Odourless	28.0± 0.05	6.51± 0.13
WL 10	Colourless	Odourless	26.9± 0.04	6.89± 0.12
WL 11	Colourless	Odourless	27.3± 0.15	7.25± 0.05
WL 12	Cloudy	Metallic	28.5± 0.17	6.49± 0.02
WL 13	Colourless	Odourless	27.1± 0.14	6.95± 0.07
WL 14	Cloudy	Metallic	28.9± 0.04	6.36± 0.01
WL 15	Colourless	Odourless	26.3± 0.01	6.81± 0.03
WL 16	Cloudy	Metallic	27.5± 0.12	6.39± 0.16
WL 17	Colourless	Odourless	26.2± 0.01	6.89± 0.18
WL 18	Colourless	Odourless	26.3± 0.04	6.54± 0.12
WL 19	Colourless	Odourless	26.6± 0.11	7.23± 0.09
WL 20	Cloudy	Metallic	27.1± 0.03	6.21± 0.01
WL 21	Colourless	Odourless	26.3± 0.12	6.67± 0.06
WL 22	Cloudy	Metallic	28.1± 0.05	6.28± 0.08
WL 23	Colourless	Odourless	28.3± 0.18	7.70± 0.05
WL 24	Colourless	Odourless	26.3± 0.17	6.88± 0.12
WL 25	Cloudy	Metallic	26.9± 0.03	6.29± 0.18
WL 26	Colourless	Odourless	26.3± 0.09	6.82± 0.15
WL 27	Cloudy	Metallic	26.1± 0.15	6.31± 0.11
WL 28	Colourless	Odourless	26.3± 0.12	7.45± 0.01
WL 29	Colourless	Odourless	26.3± 0.04	7.87± 0.17
WL 30	Colourless	Odourless	26.3± 0.15	6.91± 0.02
WL 31	Cloudy	Metallic	27.5± 0.01	6.33± 0.04
WL 32	Colourless	Odourless	26.3± 0.14	7.11± 0.11
WL 33	Colourless	Odourless	26.3± 0.12	6.62± 0.13

Note: Results are mean and standard deviation of triplicate analysis Key: WL= Well water; SD= Standard deviation

be present in drinking-water.

Bacteria contaminate water samples through inadequate treated sewage or runoff of organic material from pastoral farmlands and presence of domestic animals in the environment is also an important contribution to the production of organic material. Thus, high incidence rate of the Enterobacteriaceae in well water samples is an indication of poor hygiene practices associated with environments dominated with local people because these bacteria are used to identify an unsanitary water supply and indicate the risk of exposure to water-borne disease. Based on the result obtained the following recommendations are made; well water should be properly boiled before using for domestic purposes and a properly protected well should be made by extending the well casing above the ground surface.

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