

The Prevalence of Bacterial Isolates in Borehole Water in Selected Communities of Emohua Local Government Area of Rivers State, Nigeria

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Abstract

Access to an improved source provides a measure of sanitary protection but does not ensure water is free of faecal contamination nor is it consistent between sources types or settings. International estimates therefore greatly overstate the use of safe drinking water and do not fully reflect disparities in access. An enhanced monitoring strategy would combine indicators of sanitary protection with measures of water quality. This investigated the prevalence of bacterial isolates on borehole waters in selected communities of Emohua local government area of River State, Nigeria. Bacterial isolates were identified based on cultural morphological characteristics and biochemical tests. The total heterotrophic bacteria count for the samples ranged from $1.0-6.0 \times 10^2$ cfu/ml were low compared to the EPA and World Health Organisation standard of 1.0×10^2 cfu/ml for total heterotrophic bacteria count. Total coliform bacteria present in the samples had ranges from 0-180 MPN Index/100mL. The study therefore recommends that awareness should be enacted in order to educate the general public on the potential health implication associated with the consumption of poor water quality and proper treatment plan of the borehole water from these selected communities should be conducted regularly to prevent public health hazards.

Keywords: Microbial contamination, Coliform bacteria, Borehole water, Micro-organism, Bacterial isolates.

Introduction

Microbial water quality verification include microbiological testing. In most cases, it involves the analysis of faecal indicator microorganisms, but in some circumstances, it may also include assessment of specific pathogen densities. Verification of the microbial quality of drinking water may be undertaken by the supplier, surveillance agencies or a combination of the two Approaches to verification include testing of source water, water immediately after treatment, water in distribution systems or stored household water. Verification of the microbial quality of drinking water includes testing for *Escherichia coli* as an indicator of faecal pollution. *E.coli* provides conclusive evidence of recent faecal pollution and should not be present in drinking-water. In practice, testing for thermotolerant coliform bacteria can be an acceptable alternative in many circumstances. While *E. coli* is a useful indicator, it has limitations. Enteric viruses and protozoa are more resistant to disinfection; consequently, the absence of *E.Coli* will not necessarily indicate freedom from these

organisms. Under certain circumstances, it may be desirable to include more resistant microorganisms, such as bacteriophages and/or bacterial spores. Such circumstances could include the use of source water known to be contaminated with enteric viruses and parasites or high levels of viral and parasitic diseases in the community. Water quality can vary rapidly, and all systems are subject to occasional failure.

In most rural communities, water is consumed without testing its portability and the major sources of water apart from rainfall are surface and river water; in some cases, borehole water.

Findings show that the water from untreated boreholes does not meet the World's Health Organisation Standards for drinking water and should be treated or boiled and filtered before drinking (S.N. Ibe *et al.*, 2005).

Some of the water contaminants were coliforms/bacterial pathogens such as *Salmonella* and, *Shigella* spp were recognized pathogens from faecal sources such as *Vibrio cholerae* (Szewzyk *et al.*, 2002). The essence of this study was to investigate the bacteria quality of water of some of the selected communities in Emohua Local government area where wells and borehole waters are sources of drinking water. The study therefore examined the prevalence of bacteria in borehole water in selected communities in Emohua Local Government Area of Rivers State in Nigeria.

Isolation and identification of the isolates showed that *Bacillus* spp was the most isolated with an occurrence of 7 (23.3%). Other organisms isolated includes *Staphylococcus aureus*, *Shigella* spp, *Salmonella* spp, *Enterobacter* spp, *Proteus* spp and *Escherichia coli*. Most of these organisms are Gram Negative microorganisms and are usually associated with gastrointestinal illness.

Water samples from four different boreholes within Uli in Anambra State, Nigeria, were collected for five consecutive weeks for bacteriological analysis to assess the portability. The range of the means was from 1.5×10^2 to 5.9×10^4 cfu/ml-1 for total aerobic bacterial load, 9 to 136 MPN per 100ml for total coliforms. The highest counts were consistently found in the samples from Cargramento Lodge, where the borehole was located in an unsanitary environment, near a pit latrine. *Enterobacter* sp., *Pseudomonas* sp., and *Staphylococcus aureus* were isolated from the samples.

Statement of the Problem

There is growing concern about the quality of drinking water due to microbial contamination found in drinking water in many places all over the world. IN Nigeria, the quality of most sachet water is below the stipulated standard of world health organization owing to the fact that government no longer provide treated pipe borne water for the general public. Available water has a doubtful quality and therefore poses a risk to the health of the people (WHO, 2011).

The essence of this study therefore was to investigate the quality of water in the selected communities, if they meet the standard for drinking. In doing so, coliform bacteria was used

as indicators. The study therefore examined the prevalence of coliform bacteria in borehole in the areas of study.

Materials and Methods

Study Area

This study was carried out in three communities in Emohua Local Government Area, These comprises of Oduoha, Isiodu and Alimini. Their coordinates range from latitude, $4^{\circ}55'0''$ to $5^{\circ}0'0''$ N and longitude $6^{\circ}45'0''$ to $6^{\circ}55'0''$ E (fig 1). The communities major source of livelihood includes farming, fishing and trading. The vegetation type is tropical rainforest. Although Choba part of the new Calabar River is a source of water to the community, individuals depend on underground water (borehole) as their source of cooking and drinkable water because the river has been reported to be polluted (Woke *et al.*, 2013). The average temperature of the area was between 22°C and 29°C with high humidity and rainfall. The major occupation of the people is farming with a few other members of the communities engaging in trading and public service.

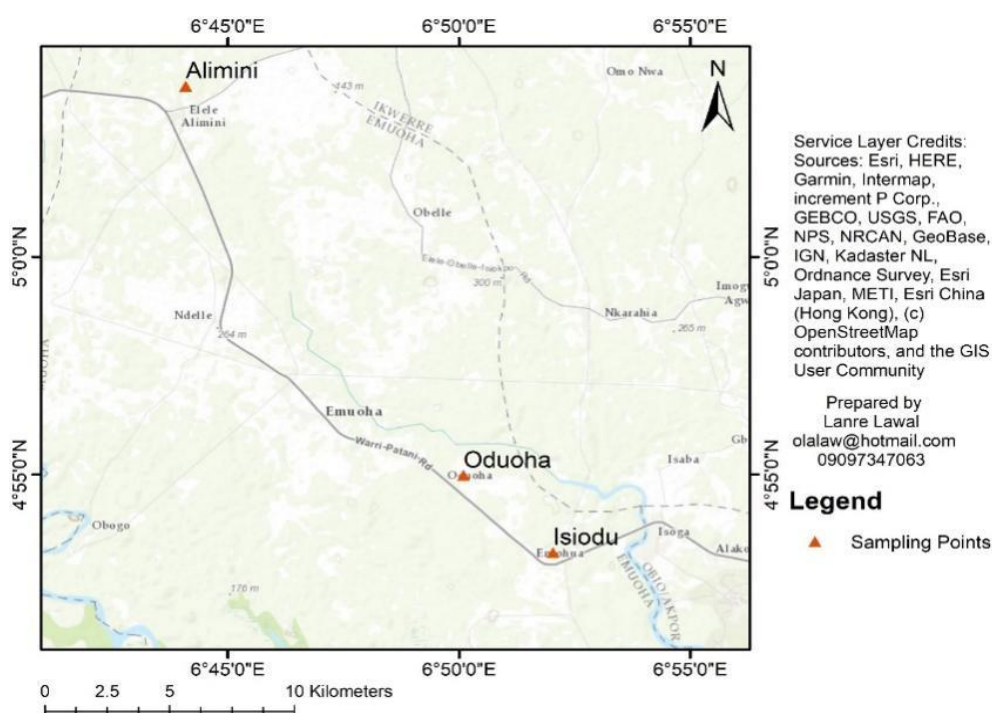


Fig 1: Study Area Map, showing the communities

Source: Researcher's Fieldwork

Sample Collection

Ten (10) borehole water samples each were collected from the three (3) communities in Emohua Local Government Area in Rivers State. The taps of the borehole water were sterilized with cotton wool soaked in 70% (v/v) ethanol. The taps were allowed to run for 2-3 minutes before sterile 250mL screw capped glass bottles were carefully uncapped and

filled with water samples and then transported to the laboratory in a cooler containing ice pack for bacteriological analysis within two hours of collection (Cheesbrough, 2006).

Growth and Enumeration of Bacteria

Culture media were reconstituted and sterilized according to manufactures instruction
Determination of total heterotrophic/Viable bacterial counts: The total bacterial, coliform, faecal coliform counts were determined thus; The coliform counts were determined by the most probable number (MPN) technique.

Nutrient Agar for Determination of Total Heterotrophic Bacteria Count

The medium was prepared by weighing 2.8g of Nutrient agar and transferred into 250ml conical flask and 100ml of distilled water was added. The media was swirled to dissolve thoroughly. Sterilization was achieved at 121°C for 15 minutes using the autoclave. The medium was allowed to cool to 45°C and 15-20 ml of the medium was poured into sterile petri dishes. The plates were allowed to solidified and then dried in an oven before use.

Determination of Heterotrophic Plate Count/Total Viable Count Using Dilution Plate

Heterotrophic plate count of all water samples was determined using dilution plate method technique on nutrient agar medium. Serial dilution of 10^{-1} - 10^{-4} were prepared using physiological saline and one ml of dilution was transferred to a sterile, empty petri dish containing nutrient and was mixed thoroughly by rotating the plate several times. The plates were inverted and incubated at 35°C for 48 to 72 hours. Following the appropriate length of incubation, 10^{-3} dilutions were selected and the visible colonies were expressed as colony forming unit per ml (Cheesebrough, 2006).

Determination of Total Coliform counts: This was done by plating a loopfull of positive MacConkey broth on Eosine Methylene Blue (EMB).

a) MacConkey Agar for Enumeration of Total Coliforms:

A mass of 4.7g of MacConkey agar was weighed and dissolved in 100ml of distilled water in a 250ml conical flask. The media was swirled for thorough mixing. It was sterilized for 15minutes at 121°C in an autoclave for 15 minutes then allowed to cool and 15-20ml was poured into the sterile petri dishes. They were sterilized in an oven before use after setting (TM-Media).

b) Eosin Methylene Blue Agar for Enumeration of *E.coli* (Faecal Coliforms):

3.6g of EMB agar was weighed with a digital weighing balance, dissolved in 100 ml of distilled water in 250ml conical flask. The media was swirled thoroughly. Sterilization was done at 121°C for 15minutes and was allowed to cool to 45°C and 15-20 ml of medium was poured into sterile petri dishes. The plates were allowed to solidify and then dried in an oven before use (TM-Media).

c) MacConkey Broth for Enumeration of Faecal Coliform (*E. coli*):

4.0 g of the MacConkey agar was weighed with a digital weighing balance, dissolved in 100 ml of distilled water in 250ml conical flask. A membrane filter paper was carefully placed with the grid side uppermost on Eosin methylene blue plate. Duplicate plates were prepared and incubation was carried out in an inverted position at 28 °C for 48 hours after which the faecal coliform bacteria that grew were counted, sub-cultured and stored on nutrient agar slants for characterization and identification.

Characterization and identification of the Bacterial Isolates

The isolates were characterized on the basis of their morphology and biochemical characteristics using Gram staining, catalase, coagulase, motility, oxidase, citrate utilization, indole, vogesproskauer, methyl red, urease and sugar (glucose, lactose and sucrose) fermentation tests as done by (Onurah *et al.*, 2019) using cultural, microscopic examination which includes sugar fermentation tests were done to identify the pure isolate as described (Cheesebrough *et al.*, 2013).

Comparison of the Prevalence of Bacterial Isolates in Each Selected Locations

This was carried out using a double strength MacConkey broth as in the above and prepared according to manufacturer's instructions and dispensed into the MacCater bottles containing Durham tubes and allowed to cool before incubating with water samples. Also Eosin Methyl Blue (EMB) Agar was used to confirm the organism in the positive tubes obtained from the presumptive test.

Results

Characterization and Identification of the Bacteria Isolated from Different Locations

Bacterial Isolates	Gram Reaction	Coagulase	Catalase	Oxidase	Indole	Urease	Citrate utilization	H ₂ S	Motility	Lactose	Mannitol	Sucrose
<i>Escherichia coli</i>	-	-	+	-	+	-	-	-	Motile	+		
<i>Klebsiella spp</i>	-	-	+	-	-	-	-	-	Non motile	+		
<i>salmonella Styphymurium</i>	-	-	+	-	-	-	-	-	motile	-		
<i>Shigella spp</i>	-	-	+	-	-	-	-	-	Non motile	-	+	-
<i>Pseudomonas aeruginosa</i>	-	-	+	+	-	-	+	-	motile	-		
<i>Staphylococcus aureus</i>	+	-	+	-	-	-	+	-	Non motile	+		

Proteus spp	-	-	+	-	+	+	-	-	motile	-
Coagulase negative staph	+	-	+	-	-	-	-	-	Non motile	
Micrococcus	+	-	+	-	-	-	-	-	Non motile	

Keys: C₀Ns - ++++++Coagulase Negative Staphylococcus

+ = Positive, - = Negative

Motility Test = Motile and Non motile

Motility ⇔ + Motile; - Non motile

The Determination of the Prevalence of Bacterial Isolates within the Selected Locations in Emohua Local Government Area

In Oduoha location, *Klebsiella*spp and *Pseudomonas aeruginosa* had the highest prevalence of 22.2% followed by *Escherichia coli* 17.0%, *Staphylococcus aureus* 11.1% and others had 5.6% each respectively (fig 3).

In Isiodu with total bacterial isolates of 15 in Isiodu *Proteus*spp, *Salmonella typhimurium* had the highest prevalence of 4 (26.7%) while *Staphylococcus aureus*, *Micrococcus* sp had the least prevalence of 1(6.7%) (fig 4).

Of the total bacterial isolates of 21 in Alimini location, *Escherichia coli* was found to have the most prevalent of 5(23.8%) and coagulase negative staphylococcus occurred as the least bacterial isolate of 1 (4.8%) (fig 5).

Prevalence of Bacterial Isolates from Selected Locations in Emohua L.G.A. in Rivers State, as in (table 4 below).

Bacterial Isolates	Selected Locations			Total Prevalence (%)
	Oduoha	Isiodu	Alimini	
<i>Escherichia coli</i>	3(17.0)	3(20.0)	5(23.8)	11(20.4)
<i>Klebsiella</i> spp	4(22.2)	2(13.2)	3 (14.3)	9(16.60)
<i>Salmonella typimurium</i>	1(5.6)	4(26.7)	3(14.3)	8(14.8)
<i>Shigella</i> spp	1(5.6)	-	-	1(2.0)
<i>Proteus</i> spp	1 (5.6)	4(26.70	4(19.0)	9(16.6)
<i>Pseudomonas aeruginosa</i>	4(22.2)	-	2(9.5)	6(11.1)
<i>Staphylococcus aureus</i>	2(11.1)	1(6.7)	3 (14.3)	6(11.1)
Coagulase negative Staphylococcus	1(5.6)	-	1(4.8)	2(3.7)
<i>Micrococcus</i> spp	1(5.6)	1(6.7)	-	2(3.7)
Total Bacterial Isolates per Selected location(%)	18(100)	15(100)	21(100)	
Total Prevalence (%)	18 (33.3)	15(27.8)	21(38.9)	54(100)

Source: Researcher's Fieldwork

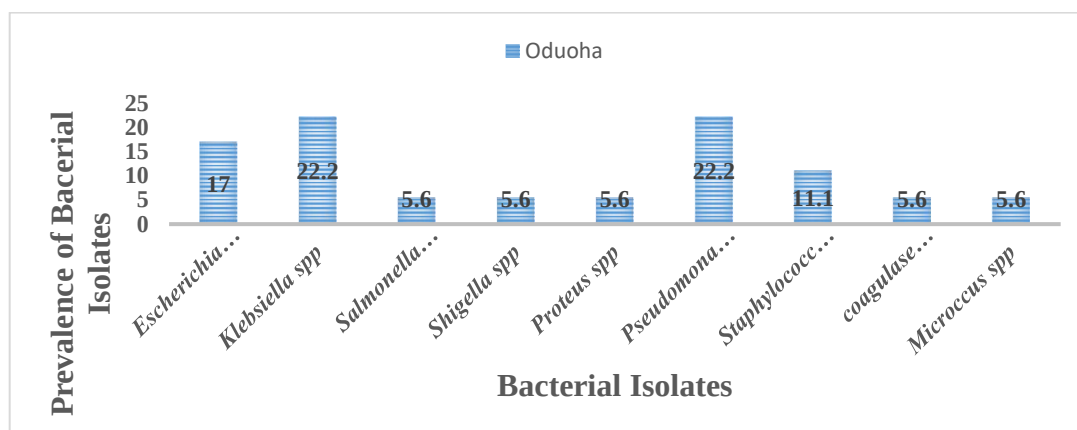


Fig 2: Prevalence of Bacterial Isolates Present in Borehole Water in Oduoha.

Source: Researcher's Fieldwork

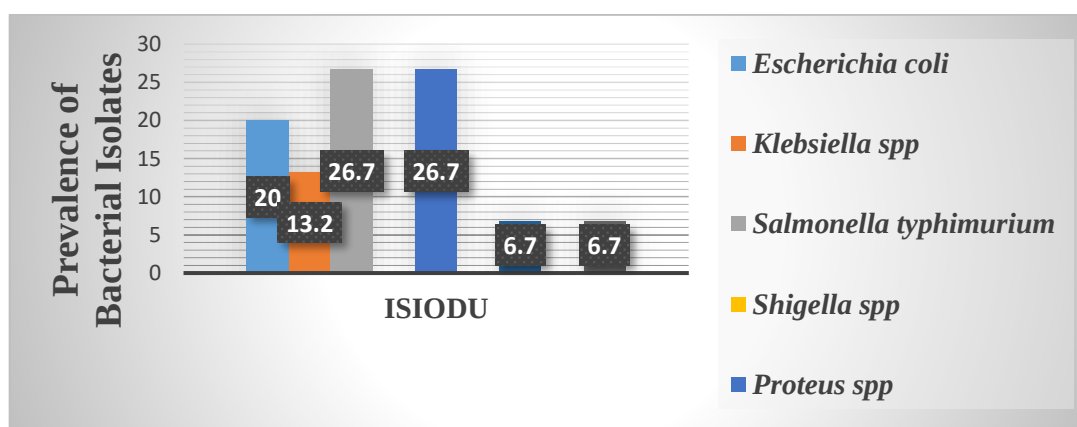


Fig 3: Prevalence of Bacterial Isolates Present in Borehole Water in Isiodu.

Source: Researcher's Fieldwork

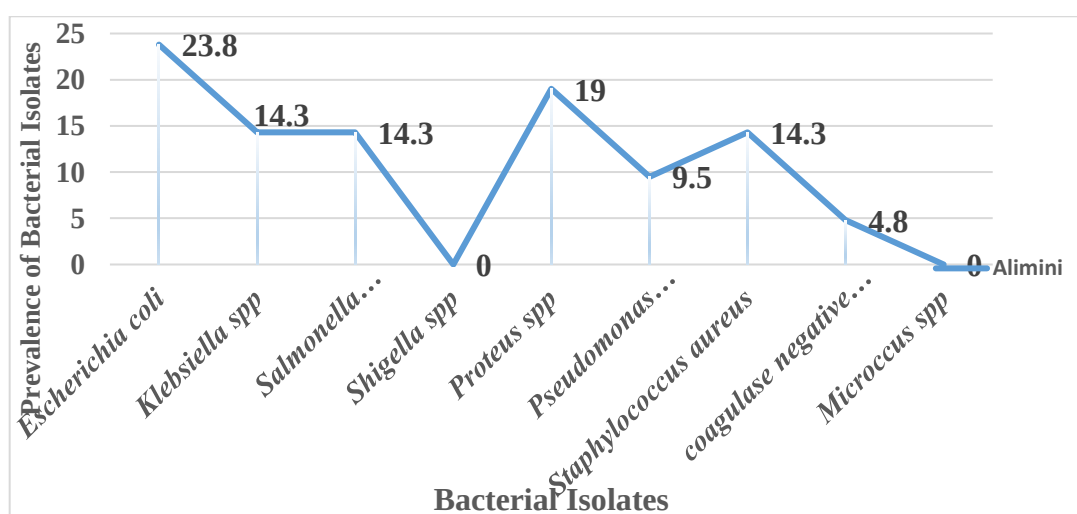


Fig 4: Prevalence of Bacterial Isolates Present in Borehole Water in Alimini Location.

Source: Researcher's Fieldwork

Comparism of the Prevalence of Bacterial Isolates in Each Selected Locations

In Odoha location, *Klebsiella* spp and *Pseudomonas aeruginosa* had the highest prevalence of 22.2% followed by *Escherichia coli* 17.0%, *Staphylococcus aureus*, *Micrococcus* sp had the least prevalence of 1(6.7%) (fig 4). Of the total bacterial isolates of 21 in Alimini location, *Escherichia coli* was found to have the most prevalence of 5(23.8%) and coagulase negative *Staphylococcus* occurred as the least bacterial isolate of 1(4.8%) (fig5). The comparism of bacterial isolates from selected locations such as Alimini, Odoha and Isiodu shows that Alimini had the highest prevalence with bacterial isolates of 38.9% followed by Odoha with the prevalence of 33.3%, Isiodu with 27.3% respectively (fig 2).

The bacterial isolates and their prevalence are shown in Table 3, with *Escherichia coli* 20.4% followed by *Klebsiella* spp and *Proteus* spp 16.6%, *Salmonella typhimorium* 14.8%, followed by *Psuedomonas aeruginosa* and *Staphylococcus aureus* 11.1% while Coagulase Negative *Staphylococcus* and *Micrococcus* spp had 3.7% and *Shigella* spp 2.0% respectively.

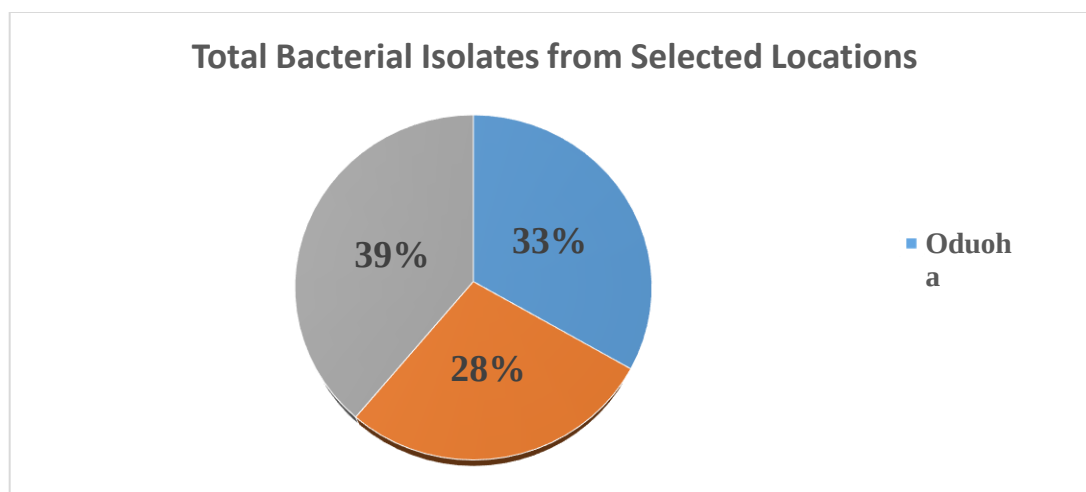


Fig 5: the comparison among the bacterial isolates in the selected location in Emohua LGA
Source: Researcher's Fieldwork

Discussion

In developing countries such as Nigeria, the majority of people live in rural areas. Water from rivers, streams, wells and more recently, boreholes serve as the main sources of water for drinking and domestic use (Ibe and Okplenye, 2005). Conformation with physicochemical and microbiological standard is of special interest because of the capacity of water to spread diseases within a large population.

Microbial Quality of well water in upland and Riverine Communities of Rivers State, Nigeria by (Chika et al.,2019) conforms with this study. The results of this study also align with similar researches by other researchers who found out that the sources of heterotrophic bacteria present in water are from runoffs, pasture, sewage, other unhygienic practices as well as animal and human wastes (Edema et al.,2001; Ibe and Okplenye, 2005; Kiman-Murage et al., 2007).

WHO (2003), specified that drinking water should be devoid of total coliform in any given sample. It is remarkable to mention that 16.7% of the water samples have zero total coliform count. The total coliform counts of the samples analyzed for this study ranged from 0-180 MPN Index/100 ml; this did not meet the acceptable standard of World Health Organisation limit of zero coliform/100ml. Previous studies in other parts of the country reported similar bacterial burden indicative of poor water quality; results of total coliforms obtained in this study is similar to Rogbesan et al., (2002), who also obtained total coliform outside the range allowed by World Health Organisation.

Woke and Bolaji in 2015 had ranged from 15-460 cfu/ml for faecal coliform count for Assessment of Ground Water Quality in Emohua L.G.A., Rivers State, Nigeria, while total coliform count ranged from 7-43 cfu/ml by Idibie *et al.*, (2018) for their study of comparative microbial analysis of borehole water and other sources of water in Benin Metropolis, Edo State. Obioma *et al.* (2017) studied the evaluation of bacteriological quality of surface, well, borehole and river water in Khana L.G.A. of Rivers State, Niger Delta and Onuorah et al., (2019), reported Bacteriological Quality Assessment of Borehole Water in Ogbaru Communities, Anambra State, Nigeria, to have total coliform count above the acceptable limit of World Health Organisation.

Microbial quality, diversity and antibiotic susceptibility profiles of bacterial isolates from borehole water used by schools in Greater Giyani Municipality, Mopani District, South Africa by Amiduo *et al.*, (2011) showed there was an association (trend) found between the use of borewell (ground) water for washing of utensils and presence of faecal coliforms in fruit juices ($p = 0.06$). This finding was supported by a study conducted in Uyo Metropolis where, ice samples were contaminated by *E.coli*, coliforms and other pathogenic microorganisms in which the major sources of water were bore wells and deep wells (NAFDAC, 2004). Similarly, in Uli, Nigeria high faecal coliform count was detected in borehole water where boreholes were proximal to pit latrine (Ibe *et al.*, 2005). Similar bacterial profile was described in Nigeria where *Escherichia coli*, *Klebsiella spp.*, *Proteus spp.*, *Enterobacter spp.*, *Pseudomonas spp.*, and *Staphylococcus aureus* were isolated from samples from boreholes. In a study carried out in Finland, shallow ground water down to a depth of 16.2m on average contained more biomass and cultivable microorganisms than did deep ground water, except in a zone at a depth of approximately 300m where the average biomass and number of cultivable microorganisms approached those of shallow water.

Epidermological investigations have proved that diarrhea has a worldwide occurrence and accounts for 4% deaths and 5% of health loss to disability. A comparative study of the physicochemical and bacteriological qualities of some drinking water sources in Abuja, Nigeria was reported by Sadiya Atiku et al., (2018). *Escherichia coli* is an organism with sewage characteristics that provide evidence for potentially dangerous pollution and so must be identified (Hutchinson, 1994). This study is in contrast with some studies carried out elsewhere. Ugbara and Otokunefor in 2015, had *Enterobacter aerogenes* as the most

prevalent followed by *E. Coli* while Idibie *et al.*, (2018) had *Enterobacter aerogenes* and *Staphylococcus aureus* as the most isolated bacteria from the borehole water samples. *Enterobacter aerogenes* can be found in vegetation and soil which serves as sources by which pathogens enter the water source (Benka-Coker and Ohimain, 1995). *Staphylococcus aureus* was also reported in this study with 11.1% of total prevalence. *Pseudomonas aeruginosa* was the most prevalent bacteria isolate from borehole water study conducted by Foka *et al.*, (2018). This organism is an important opportunistic biofilm forming pathogen associated with contaminated domestic plumbing systems (Bagh *et al.*, 2004; Penna *et al.*, 2002). *Pseudomonas aeruginosa* was also isolated in this study with 11.1% of total bacteria isolates.

Conclusion

Theodor Escherich *E.coli* was widely distributed in the intestine of humans and warm blooded animals and is the predominant facultative anaerobe in the bowel and part of the essential intestinal flora that maintains the physiology of the healthy host. *E .coli* are not pathogenes but regarded as opportunistic pathogens that cause infections in immuno compromised hosts, when ingested causes gastrointestinal illness in healthy humans. In 1914, the US Public Health Service adopted the enumeration of coliform as a more convenient standard of sanitary significance. Awareness programs in order to educate the general public on the potential health implication associated with consumption of poor water quality. Proper treatment plan of the borehole water from these selected communities should be conducted regularly to prevent public health hazards. Awareness programs in order to educate the general public on the potential health implication associated with consumption of poor water quality. Proper treatment plan of the borehole water from these selected communities should be conducted regularly to prevent public health hazards. Access to an improved source provides a measure of sanitary protection but does not ensure water is free of faecal contamination nor is it consistent between sources types or settings. International estimates therefore greatly overstate the use of safe drinking water and do not fully reflect disparities in access. An enhanced monitoring strategy would combine indicators of sanitary protection with measures of water quality. As reaffirmed by the United Nations in 2010, access to clean water is fundamental to human health. The importance of water to human health and wellbeing is encapsulated in the human right to water.

Recommendations

1. Awareness should be enacted in order to educate the general public on the potential health implication associated with the consumption of poor water quality.
2. Proper treatment plan of the borehole water from these selected communities should be conducted regularly to prevent public health hazards.

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