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MOLECULAR IDENTIFICATION OF *Escherichia coli* ISOLATED FROM DRINKING WATER SOURCES AVAILABLE TO PEOPLE IN SELECTED VILLAGES WITHIN OYUN LOCAL GOVERNMENT AREA OF KWARA STATE, NIGERIA

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Abstract
Good quality water supply and accessibility are part of the objectives of the sustainable development goals which is aimed at ensuring environmental sustainability. Good quality water is essential for the sustenance of life. This study was aimed at molecular identification of *Escherichia coli* isolated from selected drinking water sources available to people within Oyun Local Government Area, Kwara State. The collected water samples were analyzed using the Most Probable Number Techniques and Portable Microbiology Laboratory kit. Total coliform MPN values ranged from 0.0 to 5.0 MPN/100ml, fecal coliform 0.0 to 2.0 MPN/100ml and Fecal Streptococcal 0.0 to 0.8 cfu/ml. The results obtained for viable count of drinking water sources ranged from 1.5 x 10^1 to 2.8 x 10^2 cfu/ ml (*Staphylococcal* count), 1.1x 10^1 to 6.2 x 10^1 cfu/ml (*Pseudomonas* count) and 0.4 x 10^1 to 2.1 x 10^1 cfu/ ml (*Bacillus* count). The result obtained from the colilert defined substrate test and petrifilm plates confirmed the presence of the coliforms and fecal coliform (*E.coli*) in some of the samples collected. The pH values of all the water sampled ranged from 6.5 to 7.3. The turbidity values ranged from 1.5 to 2.7 NTU while the Total Dissolved Solids (Mg/L), Electric conductivity (µS/cm), Calcium ion, Fluoride ion, and Magnesium ion, of all the water sampled ranged from 48 to 75,110 to 154, 8.2 to 13.3, 0.21 to 0.35 and 2.6 to 7.1, respectively. Urgent interventions in term of adequate provision of portable water by the Government and enlightenment of the populace in the areas without portable water within the community on the risk associated with drinking contaminated water should be carried out.
Key words: *E. coli*, MPN, Portable, Microbiology, Laboratory

Introduction

Good quality water supply and accessibility are part of the objectives of the sustainable development goals (S.D.Gs) which is aimed at ensuring environmental sustainability. Good quality water is essential for the sustenance of life and monitoring its quality is of paramount importance in order to prevent disease outbreak associated with low quality water (Onyango, Okotu, Kunyang and Aliwa, 2018).

Homoida and Goja (2013) observed that one of the serious problems faced by the populace in developing countries including Nigeria is the contamination of water bodies with fecal materials, industrial sewage, domestic and agricultural wastes. The water sources such as streams, lakes, rivers and unprotected open wells are vehicles for water borne diseases which include cholera and typhoid fever. The effective treatment of water from these sources is essential to prevent or eliminate microbial contaminants (Burgess and Pletschke, 2012).

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UNICEF (2000) observed that the intervention in the water supply sector include effective rehabilitation of all existing water sources and massive drilling of boreholes in rural and semi-urban areas. Hence, incorporation of effective treatment facilities is necessary to provide portable water for both humans and animals consumption. Population increase has exerted more pressure on the water availability. Consequently, more than 1.2 billion people worldwide do not have access to safe water (Onyango *et al*., 2018).

Majority of the rural dwellers in developing countries Nigeria inclusive lack access to portable water. Seventeen (17) % of children under the age of 5 die annually as a result of diarrhea usually following the intake of contaminated water (Roch *et al*., 2016). Majorly, people residing in some villages within Oyun local government areas of Kwara State lack access to portable water supply which negatively affected them. People of these communities resulted to drinking of water majorly from unprotected water sources such as well and pond.

Statement of Problems

Inadequate availability of improved water and sanitation in our rural areas majorly contributed to the outbreak of water borne diseases leading to the increased rates of morbidity and mortality mostly among the children and immune suppressed adults.

Aim

The aim of this research was to identify the *Escherichia coli* isolated from selected drinking water sources available to people in selected villages within Oyun Local Government Area of Kwara State-Nigeria based on 16SrRNA sequencing.

Research Objectives

The main objective of this research is to assess the quality of the water supply to the people in selected villages within Oyun Local Government Areas of Kwara State so as to ascertain the potential health risk associated with contaminated water supply.

The specific objectives of this research are:

- ❖ to identify the major sources of water available to the selected communities.
- ❖ to determine the quality of the communities' water sources.
- ❖ to recommend necessary interventions in ensuring sustainable access to safe drinking water.

Materials and Methods

Surveillance Visit to the Communities

Surveillance visit was undertaken to identify communities without good quality drinking water sources within Oyun Local Government Area of Kwara State. The selected study areas include five (5) villages all within Oyun local government area. All the sources of water (hand pumped borehole and well) available to and used by each community were identified and defined as recommended by the World Health Organization (WHO, 2011).

Design and Administration of Questionnaire

A three part questionnaire was designed based on the WHO guideline for drinking water quality. The first part was focused on demographic information, the second part looked at water sourcing and the third part was based on water usage. The close ended questionnaire was administered to twenty (20) residents of the five (05) different communities where twenty (20) samples of drinking water were collected in a completely randomized manner. However, households were targeted rather than individuals. The questionnaire was directly administered and collected immediately

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Sampling and Sample Source

Samples of water from the various sources for drinking and other domestic uses were collected between April, 2024 and June, 2024 with sterile transparent whirl pack sample bag of 200 ml by volume. The samples were coded appropriately.

A total of twenty (20) samples of drinking water from different sources within the study areas were collected aseptically for analysis. The samples consisted of sixteen (16) wel, three (3) hand pumped borehole and one (1) Motorized bore hole water.

The samples were transported immediately to the Microbiology Laboratory of the Biological Sciences Department, Federal Polytechnic, Offa where they were analyzed.

Analytical Methods

The quality of water for each water source was analyzed based on the WHO guideline for drinking water (WHO, 2011).

Bacteriological Analysis

Enumeration of Viable Heterotrophic Bacteria

The population of heterotrophic bacteria in each water sample was determined by inoculating 1ml of the sample on plate count agar. Mannitol salt agar was used for the enumeration of *Staphylococcus aureus*, Centrimide agar was used for the enumeration of *Pseudomonas spp* while Tryptic soy agar and KF streptococcal agar were used for the enumeration of *Bacillus spp* and fecal *Streptococci* respectively. All these media were prepared based on the manufacturers description. Each inoculated medium was incubated at 35 ^{o}C for 24-48 hours. The bacterial counts were expressed as colony forming units per ml (cfu/ml) as described by (Onyango *et al*., 2018).

The isolates from all the media were sub-cultured separately on sterile nutrient agar plates. The plates were incubated at 35 ^{o}C for 24 hours in order to carry out morphological and biochemical tests.

Enumeration of Fecal Bacterial Indicators

The total coliform bacteria and fecal coliform bacteria were determined as most probable number (MPN) using the multiple tube fermentation test as described by Oyedun, *et al.,* (2016) The multiple tube fermentation test was performed in three steps using lactose broth medium. The steps include: (1) Presumptive test (2) Confirmatory test and (3) Completed test.

The lactose broth was weighed and dispensed in distilled water based on the manufacturer description to prepare both double and single strength media. 10ml of the double strength broth was dispensed separately in five tubes and the single strength broth in 10 tubes. The inner vial (Durham's tube) was inserted in each of the fifteen tubes in inverted position and they were examined to be full of the medium with no air bubble. All the fifteen tubes were sterilized inside the autoclave at 121 ^{o}C for 15 minutes.

Presumptive test: After sterilization of the medium, the tubes and content were allowed to cool down and inoculated with water sample. 10ml each of water sample was inoculated in the five tubes containing 10ml double strength broth using sterile pipette. One ml each of water sample was added to 5 out of 10 tubes containing single strength broth while 0.1ml each of water sample was inoculated into the remaining 5 tubes.

All the tubes were incubated at 35 ^{o}C for 24 hours. The number of positive tubes (the tubes that showed both acid production as a result of colour change in the medium and gas production as trapped

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with inverted Durham's tubes) were counted and recorded as MPN/100ml of the sample. This procedure of presumptive test was repeated to enumerate the fecal coliform (thermotolerant) bacteria of each water sample by incubating the tubes at elevated temperature of 44.5 °C for 24 hours. The results were then recorded as MPN/100ml of the sample.

Confirmatory Test: Sterilized wire loop was used to transfer 2 drops of feacal coliform culture medium from each of the fermentative tubes with presumptive positive result to:

1. Three (3) ml of lactose broth inside the fermentative tube containing inverted Durham's tubes.
2. Nutrient agar slant.
3. Three (3) ml of tryptone water. The inoculated lactose broth fermentation tube and the tryptone water were incubated at 44.5 °C for 24hiurs while the inoculated agar slant was incubated at 37 °C for 24 hours. Gram stained preparation was made from the slant and viewed under the microscope to reveal Gram-negative non-spore forming rods.

Kovacs reagent (0.1 ml) was added to the tryptone water after incubation and was gently mixed. The presence of indole was indicated by a red colour forming a film over the aqueous phase of the medium.

Completed test: The inoculum from each positive tube of the confirmatory test was streaked on plates of Eosin methylene blue (EMB) agar which had previously prepared according to the manufacturer descryption. The plates were incubated at 44.5 °C for 24 hours.

Portable Microbiology Laboratory Kit(Colilert Defined Substrate and Petrifilm plate)

Colilert Defined Substrate

The fecal indicator organism was equally assessed by inoculating 10 ml of each water sample into the tube of colilert specialized medium containing *O*-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG) as the only nutrient. The colilert tubes were incubated at temperature of 37°C for 24 hours. After the incubation period, the colilert tubes with positive coliforms growth that is, yellow colouration of the medium were observed under long wave length ultra violet (UV) light for fluorescence to detect the presence of *Escherichia coli.*

Petri film plate.

One (1) ml of each water sample was equally inoculated into the petri film plate which contains 4-methylumbelliferyl-β-D-glucuronide (MUG) as the specialized substrate. The plates were incubated at 37°C for 24 hours. After the incubation period, the *E. coli* colonies with bluish metallic sheen were counted appropriately.

Characterization and identification of Organisms

All isolates obtained were characterized based on colonial morphology, staining reaction and biochemical characteristics. The colonial morphology of the organisms were observed to reveal the shape, size, consistency, optical characteristics, pigmentation and elevation. The microscopic examination and staining techniques were carried out to determine the cellular morphology of the isolates.

Molecular Identification

The two *Escherichia coli* isolates from well B and D were identified molecularly using 16SrRNA Primer

The genomic DNA was extracted from the isolates using the Quick DNA Bacteria kit (Zymo Research Catalogue No D6005) the target region of the DNA was amplified using One tag Quick load 2xMaster mix (NEB Catalogue NoM0486) With the primer 16SrRNA. The PCR products were run on a gel. The extracted fragments were sequenced in a forward and reverse direction as described by Oyedun *et al*.(2016).

Physicochemical Analysis

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The physicochemical parameters were determined according to the procedure of Homaida and Goja (2013). The turbidity of each water sample was determined by Nephlometric method using (HACH 2000) turbidity meter and the results were reported in Nephlometric Turbidity Unit (NTU). The total dissolved solids (TDS) of each water sample was determined with (HACH 2000) TDS meter. The electric conductivity (EC) in μs/cm of each water sample was observed at 25^{o}C using conductivity meter (HACH 2000). Magnesium, fluoride and calcium were determined using titration method. All these instrument were calibrated and subsequently dipped separately into each of the water sample. All the readings were taken and recorded.

Determination of Total Hardness and Fluoride in water samples

Total hardness (Ca and Mg) and fluoride content of each water sample were determined by Ethylenediaminetetraacetic acid (EDTA) titration method as described by Homaida and Goja (2013). For the determination of total hardness, 25cm^3 of each water sample was measured into a conical flask. Two (2) cm^3 buffer solution of pH 10 was added which was followed by the addition of three (3) drops of Eriochrome Black T indicator solution. This was then titrate with 0.01N EDTA until the solution turned from red to blue. The titration was repeated to obtain two concordant results. For the determination of Calcium ion, Ca^{2+}(aq) concentration in each water sample, 25cm^3 of water sample was equally measured into a conical flask. Fifteen (15) drops of 50% w/v of sodium hydroxide (NaOH) solution was added and the mixture was swirl and left undisturbed for 2 minutes for complete precipitation of magnesium ions, Mg^{2+} (aq) as (Mg(OH)2. This was followed by the addition of 0.5g hydroxynaphthol indicator and then titrate with 0.01N EDTA until colour changed to blue. This titration procedure was repeated to obtain two concordant results (Homaida and Goja, 2013).

The determination of fluoride ion (F -) was carried out as described by Homaida and Goja (2013). One ml of Barium chloride solution ($BaCl_2$) was added to 10ml of each water sample in a conical flask which was brought to boil, cooled, immediately and filtered. 1ml alizarin solution was added as indicator. This solution was titrated with thorium nitrate solution added drop wise with vigorous stirring from the burette until the colour changed from lemon yellow to deep yellow. The titration procedure was repeated to obtain two concordant results.

Results

Sanitary surveillance of the sampling sites.

The sanitary surveillance visit made to the study areas revealed the various risk factors observed that might contribute to the poor quality of the water sources in some study areas.

Bacteriological Analysis

The results of the Total plate counts (TPC), Total coliform count (TCC), Fecal coliform counts (FCC) and Fecal Streptococcal counts (FSC) are shown on Table 1. The Staphylococcal counts were ranged from 8.2 x 10^1 – 2.8 x 10^2cfu/ml for well water, 2.5 x10^1 - 9.8 x10^1 cfu/ml for hand pump borehole while the value for the motorized borehole was 1.1 x 10^1 cfu/ml. The growth counts for *Pseudomonas* were ranged from 1.2 x 10^1- 6.2 x 10^1 and 1.1 x 10^1- 2.8 x 10^1 and 0.4x10^1 for well water, hand pump borehole and motorizes bore hole water samples respectively. The Bacilli count ranged from 0.5x10^{1-} 5.6x10^1 for well water while 0.4x10^1 was recorded for Bacilli count in sample B of the hand pump bore hole water while the motorized bore hole water sample had no Bacilli count. Total coliform MPN values were ranged from 2.0 to 5.0/100ml, 0.0 to 3.0/100ml for well and hand pump borehole water respectively while the motorized bore hole had no coliform count. For the fecal coliform MPN values, two well water samples(

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B and D) had fecal coliform Of 1.0 and 2.0 respectively. The hand pump bore hole and motorized borehole water sampled had no fecal coliform. The fecal streptococcal growth counts were ranged from $0.4x10^1$ to $0.8x10^1$ cfu/ml for the well water sampled while the hand pump bore had $0.2x10^1$ in sample A. No fecal Streptococci was found in motorized bore hole water sampled.

Table 1: The Bacteriological Examination of Drinking Water Samples from Various Sources

Sample	Staphylococcal count	*Pseudomonas* count	*Bacillus* Count	TCC MPN/100ML	FCC MPN/100ML	FSC Count
Well	$8.2x10^1$ – $2.8x10^2$	$1.2x10^1$ – $6.2x10^1$	$0.5x10^1$ – $5.6x10^1$	2.0-5.0	1.0 – 2.0	$0.4x10^1$- $0.8x10^1$
Hand Pump Borehole	$2.5x10^1$ – $9.8x10^1$	$1.1x10^1$ - $2.8x10^1$	$0.4x10^1$	0.0 -3.0	0.0	$0.2x10^1$
Motorized Borehole	$1.5x10^1$ – $3.1x10^1$	$0.4x10^1$	0.0	0.0	0.0	0.0

TCC = Total coliform count; FCC; Feacal coliform count; FSC = Feacal streptococci count;

Portable Microbiology Laboratory

Colilert defined substrate medium

The colilert defined substrate medium water analysis results corroborate with the fecal coliform (*Escherichia coli*) MPN values for well water as shown on Table 1 which revealed that some of the well water samples collected showed positive result inside the colilert tubes after incubation period by changed in colour of the medium to yellow as a result of the hydrolysis of *O*-nitrophenyl -β-D-galactopyranoside (ONPG) which released *O*-nitrophenol and subsequently, the results of the 4-methylumbelliferyl-β-D-glucuronide (MUG) modification inside the colilert tube to yield fluorescence upon observing under the UV light which indicated the presence of *Escherichia coli.*

Petri film Plate

The results obtained from the petri film plates equally revealed the presence of *Escherichia coli* in two samples out of the 16 sampls of well water collected.

Morphological and Biochemical Identification

Morphological and Biochemical identification test of the bacteria isolated revealed the presence of *Bacillus, Pseudomones, Staphlococus, Streptococcus* and *Escherichia coli* as shown on Table2

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Table 2: Morphological and Biochemical Characteristics of the Isolates

Tentative organisms	Elevation	Surface	Cons	Pig	Gram staining	Shape	Spore staining	Motility	Catalase	Oxidase	Glucose	Indole	Coag	MR	VP	Cit	Ure
E. coli	Raised	Smooth	Mucoid	Greyish white	- ve	Short rod	- ve	+ ve	+ve	-ve	+ve	+ve	-ve	+ ve	-ve	-ve	-ve
Bacillus	Flat	Rough	Mucoid	Yellow	+ ve	Rod	+ ve	+ ve	+ve	-ve	+ve	-ve	+ve	- ve	+ve	+ve	- ve
Staphlococcus	Convex	Smooth	Soft	Golden yellow	+ ve	Sphere (cluster)	- ve	- ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve
Pseudomonas	Flat	Rough	Mucoid	Blue green	- ve	Rod	- ve	+ ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
Feacal Streptococcus	Raised	Rough	Mucoid	Dark red	+ ve	Sphere (chain form)	- ve	- ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve

Key: MR: Methyl red, VP: Voger proskauer, Cit.: Citrate, Ure: Urease, Pig: Pigmentation, Cons: Consistency, Coag.: Coagulase - ve = Negative, + ve = Positive

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Physicochemical Parameters

The results for the physicochemical parameters of the various water sources revealed that the pH values of all the water sampled from different villages ranged from 6.5 – 7.3 wich conform with WHO standard pH value of (6.5-8.5). The turbidity values of all the water samples ranged from 1.5 to 2.7 which also conform with the WHO standard of drinking water which should not be greater than 5.0 NTU.

The total dissolved solids (TDS) mg/l, Electric conductivity (EC) μs/cm, calcium ion (Ca^{2+}), fluoride ion (F^{-}) and magnesium ion (mg^{2+}) values of all the twenty (20) water sampled are within the limit of WHO standard. The WHO maximum permitted level are stated respectively as 500mg/l, 1000 μs/cm, 75mg/l, 1.5 and 30mg/l.

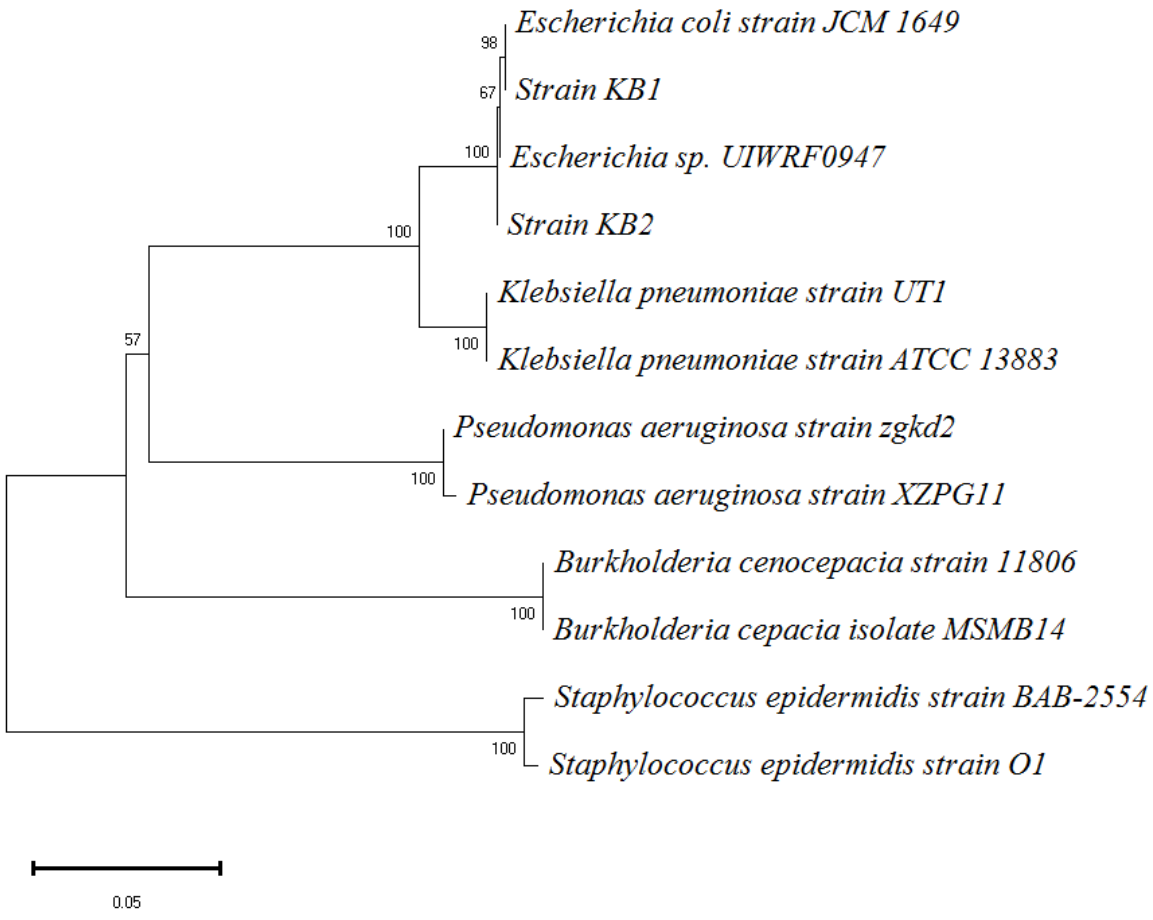


Figure 1: Phylogenetic tree constructed by Neighbor-Joining method derived from analysis of the 16SrRNA gene sequences of selected isolates and related sequences obtained from NCBI. Scale bar, 0.05 substitutions per nucleotide position.Molecular

Molecular Sequencing of JCM1649 and UIWRF0947 Strains of *Escherichia coli*

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ATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGTAACAGGAAGCAGCTTGCTGCTTTGCTG
ACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCNGATGGAGGGGGATAACTACTGGAAACGGTA
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