MOLECULAR CHARACTERIZATION AND ANTIBIOGRAM OF SELECTED PATHOGENS ISOLATED FROM STREAMS AND WELLS WATER SOURCES IN THREE LOCAL GOVERNMENT AREAS OF RIVERS STATE.

ABSTRACT

Aims: Reliance on unhealthy water sources such as hand dug wells and streams have led to the occurrence of waterborne diseases such as amoebic dysentery, gastroenteritis, typhoid fever and cholera among several communities in Rivers State. This study tends to identify selected multidrug resistant pathogens isolated from streams and wells water sources in three Local Government Areas of Rivers State using molecular techniques

Methodology: A total of 48 well and stream water samples were collected aseptically from 24 sampling sites in Ikwerre, Emohua and Etche Local Government Areas of Rivers State. Samples were analysed using membrane filtration technique for total heterotrophic bacterial count and total coliform count. The well and stream water samples were also cultured for E*. coli, Salmonella* and *Vibrio* sp. Antibiotic sensitivity test of the isolates was carried out using the disk diffusion method and multidrug resistant isolates were identified using PCR-based techniques (16S rRNA)

Results: The results of this study showed that total aerobic heterotrophic bacteria count occurred more in Emohua LGA for both well and stream water sources with bacterial count of 1.71 $x10^4$ cfu/ml for well water samples and 2.35 x 10^4 cfu/ml for stream water samples. The mean fungal count of the stream water samples was highest in Emohua LGA with 2.55 x 10^4 cfu/ml followed by Ikwerre LGA which had 4.20 x 10^3 cfu/ml and Etche LGA with the least count of 1.80 x 10^2 cfu/ml. All the isolated bacteria species were found to be resistant to more than 50% of the antibiotics used with highest Multi Antibiotic Resistant Index of 0.625. multidrug resistant bacterial isolates identified include *Vibrio* sp strain 201707CJKOP-Y162 (MG593726), *Bacillus sbtilis* clone N55 (JQ622582), *Chryseobacterium* sp strain CB2915-325-DE_0621 (MH512534), *Enterobacter* sp strain AB55 (MF407145), *Klebsiella pneumonia* strain M792-16(MH680830), *Shigella sonnei* strain AR_0030(CP032523), *Escherichia coli* strain WECHEC025943(CP027205) and *Salmonella bongori* strain KC153129.116 (MG663494)

Conclusion: Access to good quality or potable drinking water and efficient sanitary practices are fundamental to human health and economic development. The occurrence of multidrug resistant pathogenic bacterial isolates in natural water sources requires serious routine evaluation in order to forestall the outbreak of waterborne disease epidemics.

Keywords: 16S rRNA; Public Health, Multidrug resistance; Stream and well water; Waterborne diseases

INTRODUCTION

Drinking water, defined as "suitable for human consumption", is free of disease-causing microorganisms. The possible consequences of microbial contamination for health are such that its control must be a primary objective and must never be compromised. Developing countries continue to struggle with the issue of food security, that is, the amount of food sufficient for consumption by the growing population; there is another dilemma in these countries. It is estimated that more than 200 types of diseases caused by pathogens are foodborne and waterborne causing problems in vulnerable groups of people such as, the elderly, pregnant women and young children. Therefore, ensuring the safety of food is an important challenge for public health [1].

Drinking water has been a major issue in many countries like Nigeria and majority of the rural populace in Nigeria do not have access to portable water [2]. Only few people can afford and rely on purified and treated water particularly for consumption therefore, underground water (well water) serve as

the major source of both drinking water and domestic water used in local population of Nigeria [3]. Majority of the disease-causing microorganisms transmitted via drinking water are predominantly faecal in origin and are commonly called enteric pathogens, Olatunji *et al.* [4]. With regards to the World Health Organization [5] standards, drinking water should not contain any microorganisms known to be pathogenic or any bacteria indicative of faecal pollution. The use of water contaminated with domestic and industrial waste, human and animal excreta leads to the development of waterborne diseases like gastroenteritis, typhoid fever, cholera, diarrhea and bacillary dysentery [6].

There is a growing public health interest relating to the uses of water and the outbreak of waterborne diseases. This concern is genuine and threatening considering the fact that water contaminated with pathogenic microorganisms when consumed or used in the preparation of food also leads foodborne disease. Rural communities in many developing countries obtain drinking water from untreated sources. In Nigeria for instance, water supply infrastructures are either still at the development stage or are totally absent in the rural communities [7]. According to a survey by Majuru *et al.* [8], about 6.5 million Nigerians have no access to safe source of water. The situation is made worse in the rural areas where only 24% of the population had access to safe water. So, the provision of clean, reliable and potable water in rural areas remains a major challenge considering the fact that majority of members of the population reside in the rural areas. When provision of clean and safe water is inadequate, residents usually resort to use of contaminated water sources to certify their water needs [9].

Most of the bacteria, viruses, parasites, and fungi that contaminate well and stream water comes from fecal material from humans and other animals, for example from on-site sanitation systems (such as pit latrines and septic tanks). Common bacterial contaminants include *E. coli, Salmonella, Shigella, Micrococcus* spp., *Pseudomonas* spp., *Streptococcus* spp., *Klebsiella* spp., and *Campylobacter jejuni*.

The use of antibiotics has helped a great deal in the treatment of waterborne diseases caused by the respective microorganisms. However, Stanley [10] noted that the major problem encountered in antibiotic treatment is the development of resistance by bacteria. The increasing use of antibiotics in food and animals leads to the wide spread occurrence of resistant enteric pathogens to humans. Gideon *et al.* [11] reported the antibiotic resistant profile of bacteria isolated from drinking water sources in Amai Kingdom, Delta State, Nigeria. Adekunle *et al.* [12] stated that the development of antibiotic resistance by enteric pathogens poses a great risk to citizens in areas where diarrhoea infection is problematic. Tolessa *et al.* [13] stated that bacteria are known to acquire antibiotic resistance through horizontal plasmids transfer mechanisms. Abui *et al.* [9] stated that in Nigeria and other many other developing nations, the apparent lack of potable water supplies remains a major problem in most of the communities.

The importance of the use of good quality water to satisfy the food, recreation, domestic and industrial needs of man have been emphasized in many reports including the United Nations Commission on Sustainable Development (2010) on water quality and WHO [5] guide line on drinking water quality. Although, numerous policies have been initiated and strategies adopted by different member countries of these world organisations, the problems of either unavailability, inadequate or poor quality sources of water supplies still abound and are confronting most cities and rural communities around the world. In Rivers State, some of the communities in the rural local government areas like Ikwerre, Emohua and Etche Local Government Areas are still shanties and largely underdeveloped. These communities are

very poor and basic social amenities like good quality source of water supply still remains a mirage. So, there is heavy dependence on hand-dug wells, shallow streams, rivers and creeks to provide for their water needs. Reliance on these unhealthy water sources have led to the occurrence of waterborne diseases such as amoebic dysentery, gastroenteritis, typhoid fever, cholera, mild/acute diarrhoea and bacillary dysentery among the residents of these local communities. This study tends to identify selected multidrug resistant pathogens isolated from streams and wells water sources in three Local Government Areas of Rivers State using molecular techniques.

MATERIALS AND METHODS

Study area and sampling sites

This research was carried out in selected villages in some communities from Ikwerre, Etche and Emohua Local Government Areas of Rivers State. Eight different sampling sites comprising of six hand-dug wells and 2 streams in each of the selected community from the three Local Government Areas were identified and sampled monthly for microbiological analysis. This made up a total sum of twenty-four sampling sites.

Collection of well and stream water samples

Six clean sterile glass bottles were used to collect well water samples from each of the six hand-dug wells in the community and transferred immediately into already labelled sterile two litre plastic bottle containers. Collection of stream water samples was done by entering into the water body up to the knee level and plunging the neck of the sterile glass bottle containers to about 30cm downward below the water to let the container fill with space left to allow for mixing. All the samples were collected in duplicate. Stream water samples collected with clean glass bottle containers were filtered immediately through a membrane of 0.45 µm.

Isolation of bacteria species from the samples

The APHA [14] membrane filter techniques was adopted. Using sterilized, the membrane filter disc (0.45 µm) was removed and placed on the surface of the porous support. Then, the funnel was placed and held infirm position on top of the porous support with clamps. 10ml of 10^{-2} serial dilution of water sample was transferred into the funnel and suction from vacuum pump was applied. The vacuum pump was terminated after the sample have passed through the membrane filter, and the funnel removed. The membrane filter disc was carefully removed using sterilized forceps and transferred on to the surface of freshly prepared Eosin Methylene Blue (EMB) Agar, *Salmonella Shigella* agar and Thiosulphate Citrate Bile Salts Sucrose (TCBS) agar plates. All the plates were incubated at 37^0C for 24 hours

Purification and maintenance of isolates

Each discrete colony on a petri dish was transferred using a sterile inoculating wire loop into plates containing freshly prepared Nutrient agar and incubated at 37^0C for 24-48hrs respectively. After incubation, the cultural characteristics of the isolates were recorded and compared with descriptive features contained in Holt *et al.* [15]. The isolates were then preserved on nutrient agar slants stored in the refrigerator at 4^oC for biochemical characterization and identification.

Biochemical characterization and identification of isolates

The methods of Oranusi *et al.* [16] was employed for the identification of the bacteria isolates. The biochemical tests that were used to further characterize bacteria are: catalase, methyl-red, oxidase,

citrate utilization, and coagulase and indole tests. The identities of coliforms and other bacteria were then confirmed using the identification aid outlined in Bergey's Manual for Determinative Bacteriology [15] as well as that of known taxa as described by Cheesbrough [17].

Molecular Identification

DNA extraction (Boiling method)

Five milliliters of an overnight broth culture of the selected bacterial isolate in Luria Bertani (LB) was spun at 14000rpm for 3 min. The cells were re-suspended in 500ul of normal saline and heated at 95^0C for 20 min. The heated bactererial suspension was cooled on ice and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml microcentrifuge tube and stored at -20^oC for other downstream reactions.

DNA Quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was launched by double clicking on the Nanodrop icon. The equipment was initialized with 2 ul of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestral, the upper pedestral was brought down to contact the extracted DNA on the lower pedestral. The DNA concentration was measured by clicking on the “measure” button.

16S rRNA Amplification

The 16s rRNA region of the rRNA genes of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 25 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; anealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extention, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 20 minutes and visualized on a blue light transilluminator.

Sequencing

Sequencing was done using the BigDye Terminator kit on on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 ul BigDye® terminator v1.1/v3.1, 2.25ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing condition were as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min.

Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 [18]. The bootstrap consensus tree inferred from 500 replicates [19] is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method [20].

Antibiogram Profile Test

Antibiotic sensitivity test of the isolates was carried out using the disk diffusion method described by Bauer *et al.* [21]. The Sensitive Test Agar (LAB M) medium was used. The medium was prepared according to manufacturer's instruction. The medium was allowed to cool to 50^0C and poured into sterile petri dish plates and allowed to solidify. When set, the agar plates were dried for 30 minutes at 35^0C by placing them upright in the incubator with the lid tilted. The inoculum of each isolate prepared was from an 18hour broth culture adjusted to obtain turbidity comparable to 0.5 McFarland Standard. 0.5ml of 1.175% (w/v) barium chloride dehydrate ($BaCl_2$ $2H_2O$) solution was added to 99.5ml of 1% tetraoxosulphate (IV) (H_2SO_4) acid to obtain the turbidity standard and used to standardize the bacteria inoculum. 18hours broth culture diluted with sterile saline was used to make adjustments. Sterile cotton tipped applicator was dipped into standardized bacterial solution. The swab was then used to streak the entire dried surface of the medium. The inoculated plates were incubated for 5 minutes to remove excess moisture.

The antibiotic disc (Abtek Biological Limited, Liverpool) was then placed at equidistance from each other on the plate with the aid of a pair of sterile forceps. The discs were pressed firmly onto the agar medium with the sterile forceps to ensure adequate contact with the agar. The inoculated plates were incubated for 18-24 hours at 35^0C. The antibiotics discs used for the test are; oflaxcin (5μ), gentamicin (10μ), nallixidic (30μg), nitrofurantoin (200μg), cotrimazle (25μ), amoxicillin (25μg), tetracycline (25μg) and augumentin (30μg). A ruler was used to measure the diameter of each zone of inhibition and recorded in millimeters (mm). The measurements were compared to a zone size interpretative chart [22], and the organisms were grouped as resistant, intermediate or susceptible to the test antibiotics according to the chart.

Multi Antibiotic Resistant Index (MARI)

This was carried out as described by Matyar *et al.* [23] with slight modification. MARI= resistant antibiotics ÷ total antibiotics tested. MARI value > 0.2 indicate existence of isolate(s) from high risk contaminated source with frequent use of antibiotic(s) while values $\leq$0.2 show bacteria from source with less antibiotics usage [24].

Results

The changes in the counts of the total heterotrophic bacterial population of well and stream water samples are presented in Table 1. The total heterotrophic bacterial counts ranged from 1.96 x 10^2 cfu/ml to 2.90 x 10^3 cfu/ml for Ikwerre LGA, and 2.75 x 10^2 cfu/ml in to 2.35 x 10^4 cfu/ml for Emohua LGA. While Etche had 1.93 x 10^2 cfu/ml to 3.57 x 10^2 cfu/ml. Total aerobic heterotrophic bacteria count occurred more in Emohua LGA for both well and stream water sources with bacterial count of 1.71 x10^4 cfu/ml for well water samples and 2.35 x 10^4 cfu/ml for stream water samples. The mean fungal count of the stream water samples was highest in Emohua LGA with 2.55 x 10^4 cfu/ml followed by Ikwerre LGA which had 4.20 x 10^3 cfu/ml and Etche LGA with the least count of 1.80 x 10^2 cfu/ml. However, the highest fungal load of 2.25 x 10^4 cfu/ml was obtained from well water source from Emohua LGA. Table 1 shows the mean total coliform count from well and stream water sources in Ikwerre, Emohua and Etche LGA's. the highest coliform count was recorded from well water sample obtained from Ikwerre LGA with coliform counts of 52 cfu/100ml while the least count was observed from stream water samples obtained from Emohua LGA with coliform counts of 1 cfu/100ml. From preliminary identification of the isolates, seven

genera of bacteria which include *Escherichia coli*, *Enterobacter* sp., *Salmonella* sp., *Shigella* sp., *Bacillus* sp., *Klebsiella* sp. and *Vibrio* sp were isolated from both the stream and well waters sources as presented in Table 2.

Table 3 shows the antibiogram profiles of the different test pathogens with selected types of antibiotics. While *E. coli* showed very high sensitivity to Oflaxicin (OFL), Nitroturuntoin and Nalidivic acid (NAL), it was totally resistant to Amoxilillin (AMX) and intermediate to Augumentin (AUG). On the other, *Salmonella* sp showed total resistance to Augmentin (AUG), Tetracycline (TET) and Amoxicillin (AMX) and high sensitivity to NAL, Oflaxicin, Nitrofurantoin and Gentaminin antibiotics. *Vibrio* sp were very resistant to Augmentin, Amoxicillin and Cotrimazole. It was highly sensitive to Nitrofurantoin, Nalidixic acid Oflaxicin, Gentamicin and intermediate to Tetracycline.

The identification of the selected multidrug resistant bacterial isolates to determine the strain was carried out using molecular techniques. Table 4 below presents both the quantity and purity of genomic DNA that was extracted from the selected bacterial isolates for genomic identification. The PCR reaction of the extracted genomic DNA samples revealed positive amplification of the 16S rRNA gene in all the multidrug resistant isolates when ran on 1.5% agarose gel at 120V for 20 min as showed in Figure 1. Clear bands observed in lane 1, 3-13 shows positive amplification of the extracted DNA samples with Lane N representing a 1000 base pair molecular ladder.

The obtained 16s rRNA sequence from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the isolate B8 showed a percentage similarity to other species at 99%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolate within the *Salmonella sp* revealed a closely relatedness to *Salmonella bongori* strain KC153129.116(MG663494) than other *Salmonella* sp respectively. The other organisms identified are; *Vibrio* sp strain 201707CJKOP-Y162(MG593726), *Bacillus sbtilis* clone N55(JQ622582), *Chryseobacterium* sp strain CB2915-325-DE_0621(MH512534), *Enterobacter* sp strain AB55(MF407145), *Klebsiella pneumonia* strain M792-16(MH680830), *Shigella sonnei* strain AR_0030(CP032523), *Escherichia coli* strain WECHEC025943(CP027205), according to the phylogenetic tree on Figure 1.

Table 1

Mean microbial count from streams and wells water samples in Ikwerre, Emohua, and Etche LGA's of Rivers State

Water sources	Microbial load in Ikwerre LGA (CFU/ml)			Microbial load in Etche LGA (CFU/ml)			Microbial load in Emohua LGA (CFU/ml)		
	THBC	TCC (cfu/100ml)	TFC	THBC	TCC (cfu/100ml)	TFC	THBC	TCC (cfu/100ml)	TFC
$Well_1$	2.68×10^2	43	2.58×10^2	3.57×10^2	25	1.52×10^2	3.10×10^3	20	1.25×10^2
$Well_2$	1.96×10^2	50	2.17×10^3	1.93×10^2	20	2.17×10^2	1.71×10^4	28	1.82×10^3
$Well_3$	1.34×10^3	52	3.48×10^3	2.33×10^2	22	2.67×10^2	2.53×10^3	37	2.25×10^4
$Stream_1$	3.30×10^2	11	2.63×10^2	2.64×10^2	6	2.96×10^2	3.69×10^3	1	2.55×10^4
$Stream_2$	3.88×10^2	3	4.20×10^3	2.25×10^2	3	2.48×10^2	2.75×10^2	9	1.38×10^3
$Stream_3$	2.90×10^3	8	1.89×10^3	3.85×10^2	2	1.80×10^2	2.35×10^4	6	2.76×10^3

Table 2

Biochemical characterization and identification of bacterial isolates from streams and well water samples in Ikwerre, Emohua, and Etche LGA's of Rivers State

Number of isolates	Gram's reaction	Cell morphology and arrangement	Catalase	Motility	Oxidase	Coagulase	Indole	Citrate	H_2S	MR	VP	Nitrate	Urease	Glucose	Lactose	Mannose	Sucrose	Mannitol	Maltose	Ribose	Probable organism
7	-	Rods in clusters	+	+	-	-	-	+	-	-	+	+	-	+	-	+	+	+	+	+	*Enterobacter* sp
9	-	Short rods in pairs and	+	-	-	-	±	-	-	+	-	+	-	±	-	+	-	+	±		*Shigella* sp
12	-	Rods in pairs	+	+	-	-	-	+	+	-	+	-	+	-	+	-	+	+	+		*Salmonella* sp
21	-	Rods in pairs	+	+	-	-	+	-	-	+	-	+	-	+	+	-	±	+	-	-	*Escherichia coli*
8	-	Rods in singles	+	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+	-	*Klebsiella* sp
13	-	Curved Rods	+	+	+	-	+	+	-	-	±	-	-	+	±	+	+	+	+	-	*Vibrio* sp
10	+	Long Rods in singles	+	+	±	-	-	+	±	-	+	+	-	+	±	+	+	+	+	+	*Bacillus* sp

Table 3
Antibiogram of *E. coli* isolates in water samples from Emohua Local Government Area, Rivers State.

Isolate No.	Zone of Inhibition (mm)											
	AUG	TET	AMX	COT	OFL	GEN	NAL	NIT	R (%)	I (%)	S (%)	MARI
Enterobacter sp	15I	13R	13R	12I	21S	14I	30S	20S	2(25)	3(37.5)	3(37.5)	0.25
Shigella sp	16I	17I	11R	21S	19S	131	29S	18S	1(12.5)	3(37.5)	3(37.5)	0.125
Salmonella sp	161	18I	11R	20S	23S	11R	16I	19S	2(25)	3(37.5)	3(37.5)	0.25
Escherichia coli	12R	I6I	12R	OR	20S	15S	22S	12R	3 (37.5)	1 (12.5)	1 (12.5)	0.375
Klebsiella sp	17I	16I	13R	14I	18S	16S	20S	18S	1 (12.5)	3 (37.5)	4 (50)	0.125
Vibrio sp 1	11R	18I	0R	15I	22S	13R	10R	19S	4 (50)	2 (25)	2 (25)	0.5
Enterobacter sp	12R	13R	0R	16I	19S	17S	12R	10R	5 (62.5)	1 (12.5)	2 (25)	0.625
Shigella sp	13R	15I	11R	18S	18S	22S	13R	24S	3 (37.5)	1 (12.5)	4 (50)	0.375
Salmonella sp	11R	11R	8R	19S	20S	14I	10R	18S	4 (50)	1 (12.5)	3 (37.5)	0.5
Escherichia coli	12R	13R	15I	17S	16I	11R	15I	11R	4 (50)	3 (37.5)	1 (12.5)	0.5
Klebsiella sp	15I	13R	13R	10R	17S	21S	0R	13R	5 (62.5)	1 (12.5)	3 (37.5)	0.625
Bacillus sp	12R	15I	10R	12R	15I	19S	12R	16I	4 (50)	3 (37.5)	1 (12.5)	0.5
Vibrio sp 2	12R	16I	15I	16I	21S	21S	18S	0R	2 (25)	3 (37.5)	3 (37.5)	0.25

Key:
OFL- Oflaxicin Gen-Gentamicin Nal-Nalidixic Acid NIT-Nitrofurantoin
Cot-Cotrimazole Aug- Augumentin Amx Amoxicillin TET – Tetracycline
R = Resistant, I= Immediate, S = Sensitive
WV: Vibrio species in well (no.1,2,5& 6) water samples
SV: Vibrio species in stream (no. 7 & 8) water samples

Table 4

Nanodrop quantified bacterial DNA

S/N	Isolate code	Quantity (ng/µl) of bacterial DNA
1	B1	12.7
2	B2	21.0
3	B3	8.2
4	B4	15.9
5	B5	5.6
6	B6	13.9
7	B7	6.4
8	B8	18.6

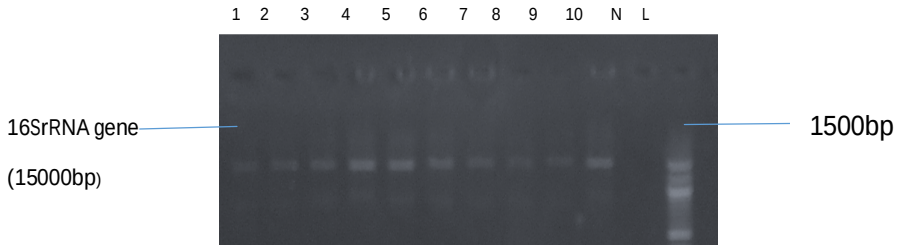

Plate 1: Agarose gel electrophoresis of the 16S rRNA gene of some selected bacterial isolates. Lanes 1, 3-12 represent the 16SrRNA gene bands (1500bp), Lane 2, failed amplification, Lane N represents the negative control, lane L represents the 1000bp molecular ladder.

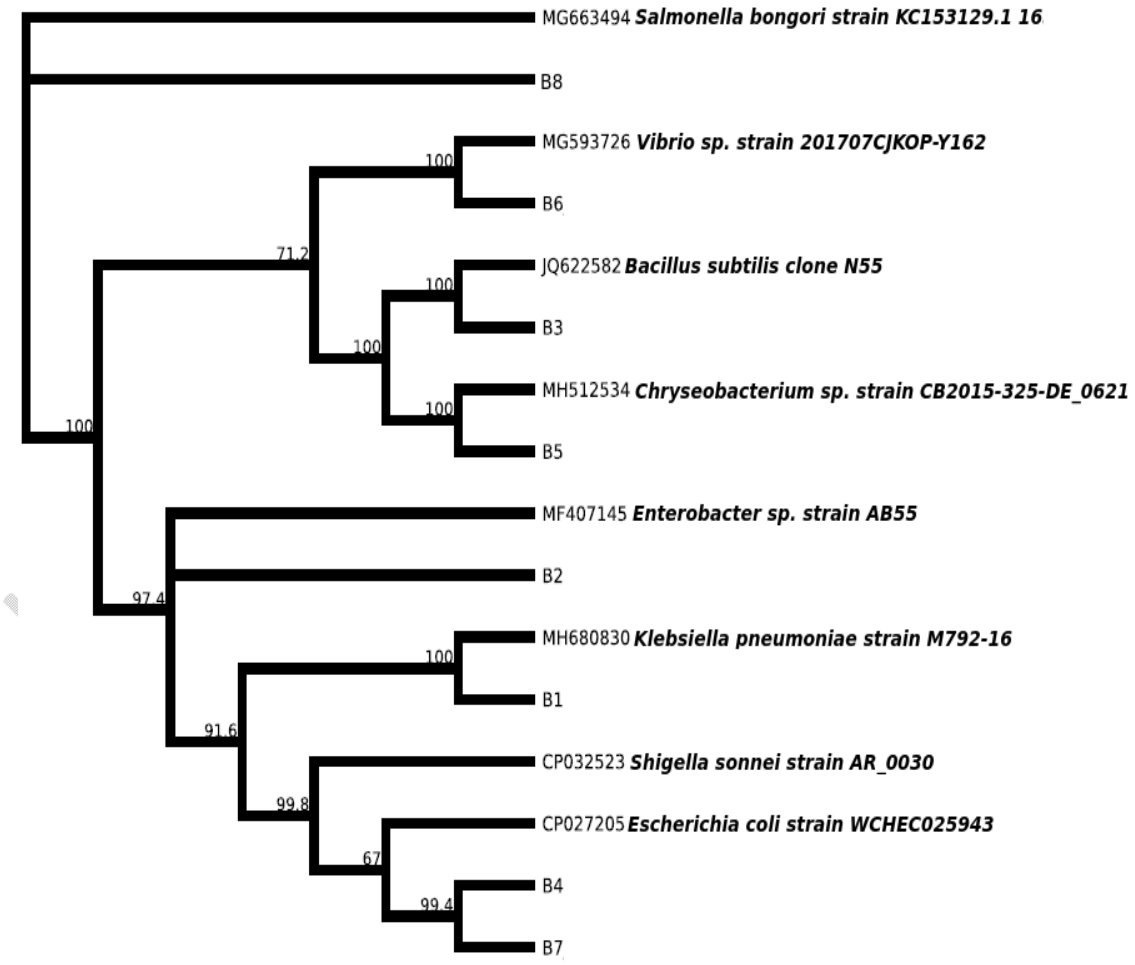

FIG.1**:** Phylogenetic tree showing the evolutionary distance between the bacterial isolates.

Table 5

Identification of the isolates by 16S rRNA genes amplification showing percentage similarities

S/N	Gene Bank isolates	Accession number	Bacteriocin positive isolates	% Similarity
1	*Klebsiella pneumonia* strain M792-16	MH680830	B1	100
2	*Enterobacter* sp. strain AB55	MF407145	B2	97.4
3	*Bacillus subtilis* clone N55	JQ622582	B3	100
4	*Escherichia coli* strain WCHEC025943	CP027205	B4	99.4
5	*Chryseobacterium* sp. strain CB2015-325-DE_0621	MH512534	B5	100
6	*Vibrio* sp. strain 201707CJKOP-Y162	JQ622582	B6	100
7	*Shigella sonnei* strain AR_0030	CP032523	B7	99.8
8	*Salmonella bongori* strain KC153129.1 16	MG663494	B8	100

Discussion

The results obtained were compared with both national and international standards. The examination of microbiological quality of drinking water sources is intended to prevent the development of waterborne illnesses or outbreaks among the rural dwellers as a result of consumption of water contaminated with harmful microorganisms [25]. Water or food items to be consumed or water that is designated for drinking should not harbour pathogenic microorganisms or any bacteria indicative of faecal contamination. The results of the mean total aerobic heterotrophic bacterial counts of the wells and stream water samples from the three Local Government Areas were found to be lower than the one reported by Anyanwu and Okoli [26] in a study of well water samples from different locations in Nsukka, Southeast Nigeria. However, Olatunji *et al.* [27] and Agwaranze *et al.* [25] in their assessment of the water quality of Asa river and well water sources in Wukari, Taraba State, Nigeria obtained a lower heterotrophic bacterial counts when compared to the present study. Although, the heterotrophic bacterial count observed in the well waters examined were high, the values were all lower than the one obtained by Shittu *et al.* [28] in their study of the Rivers and well waters in Kuta Town, Ogun State, Nigeria. Results from the total coliform bacterial count of the wells and stream water sources in this study revealed the presence of coliform bacteria in many of the wells and streams across the three LGA's. The result of this study is in line with the findings of Yahaya *et al.* [29] who reported that the contamination of water sources by coliform can be high during raining season than the dry season. This is evident when the water sources are not shielded from surface run-off during the wet season. Moreso, the grazing of domestic animals which are seen freely roaming about in the various communities of the three LGA could have also added to the pollution of the water sources.

The result of the biochemical test indicated the presence of *E. coli*, *Klebsielle* sp, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Salmonella* sp, *Vibrio* sp, *Shigella* sp, and *Bacillus* sp. Romulus *et al.* [30] also isolated *Salmonella, Escherichia coli, Vibrio, Enterobacter, Klebsiella* and *Pseudomonas from* the shallow wells in Kitui Town, Kenya. Okunye and Odeleye [31] isolated and

identified *Vibrio* sp in their bacteriological investigation of well water samples from selected market locations in Ibadan, Nigeria.

In addition to the infections caused by these organisms, another major problem associated with these pathogens is the development of drug resistance. Saif *et al.* [32] stated that antibiotic resistant bacteria can infect human via contaminated food and drinking water, or directly from the environment. According to Samantha and Janeas [33], these superbugs result in infections that are only responsive to treatment with a few if any currently available antimicrobial agent. What is worrisome, is that these resistant organisms cause waterborne infections that leads to high rate of morbidity and mortality among residents of affected communities. There is therefore, a huge healthcare cost usually expended on waterborne diseases caused by resistance pathogens.

The contamination of water sources used for household chores and drinking purposes with harmful microorganism account for a major health problem, especially among the low income group and the rural dwellers in developing countries. Microbiological analysis of the well and stream water sources for the various communities in the three local government areas investigated showed a slightly high values for bacteria and fungi count. The biochemical characterization and identification of the isolates revealed the presence of potential waterborne pathogens, such as *E. coli, Salmonella* sp, *Enterobacter* sp., *Shigella* sp., *Vibrio sp Bacillus* sp. and *Klebsiella* sp that are hazardous to human health when consumed or ingested.

CONCLUSION

Access to good quality or potable drinking water and efficient sanitary practices are fundamental to human health and economic development. The occurrence of multidrug resistant pathogenic bacterial isolates in natural water sources requires serious routine evaluation in order to forestall the outbreak of waterborne disease epidemics. Although, some progress has been made by both the local, state and federal authorities towards the provision of potable water to prevent the occurrence of waterborne disease, a lot still remains to be done to totally eradicate this deadly menance.

REFERENCES

1. Paudyal N, Anihouvi V, Hounhouigan J, Matsheka MI, Monang BS. (2017). Prevalence of foodborne pathogens in food from selected African countries-A meta-analysis. Int. J. Food Microbiol. 2017;249: 35-43.
2. Idowu AO, Oluremi BB, Odubawo KM. Bacteriological analysis of well water samples in Sagamu. Afr J Clin Exp Microbiology. 2011; 12:86-91
3. Niba RN, Chrysanthus N. Bacteriological analysis of well water sources in the Bambui student residential area. Journal of Water Resource and Protection. 2013; 5:1013-1017.
4. Olatunji JA, Odediran OA, Obaro RI, Olasehinde PI. Assessment of Groundwater Quality of Ilorin Metropolis using Water Quality Index Approach. Nigerian Journal of Technological Development. 2015; 12 (1); 18-21.
5. WHO. World Health Organization. Guidelines for Drinking-Water Quality. Microbial Fact Sheet. 2014;229-31. Available:http://www.who.int/water/gdwq3r ev/en. 23g dwq3_11.pdf G.
6. Okwelle AA, Asikong BE, Lennox JA. Bacteriological Quality Assessment of Well and Stream Water Sources from Ikwerre, Emohua and Etche Local Government Areas of Rivers State. Microbiology Research Journal International, 2021;31 (8):2456-7043

7. Rossiter HMA, Owusu PA, Awuah E, MacDonald AM, Schafer AL. Chemical Drinking Water Quality in Ghana: water costs and scope for advanced treatment. Journal of Science and Total Environment. 2010;408(11); 2378-2386
8. Majuru B, Michael-Mokoena M, Jagals P, Hunter PR. Health Impact of small-community water supply reliability. International Journal of Hygiene Environment and Health. 2011;214(2); 162-166.
9. Abui MY, Garba DP, Rikichi B, Stephen S. The Challenges of Rural water supply in Nigeria. Dynamic Journal of Environmental Science and Technology. 2016;1(4): 24-30.

   Academic Press.
10. Stanley CO. The antibiotics susceptibility pattern of *Escherichia coli* isolated from well water in Afikpo, South Eastern Nigeria. American Association Science and Technology Journal of Biology. 2015;1(3); 38-42
11. Gideon IO, Inamul HM, Alexander AO, Iftikhar AT. Antibiotic Susceptibility Profile of Bacteria Isolated from Drinking, Water Sources in Amai Kingdom, Delta State, Nigeria. Annual Research & Review in Biology. 2017;14(1); 1-9.
12. Adekunle OA, Mary AB, Oyinade AA, Bridget OF, Adebanke OA. Antibiotics sensitivity patterns of Escherichia coli and Aerobacteraerogenes isolated from well water in Ile–Ife, Nigeria. International Journal of Medicine and Medical Sciences. 2011;3(5);155-160.
13. Tolessa M, Etana D, Ofgea KC. Antibiotic sensitivity pattern of pathogenic bacterial isolates of public health concern from Lake Hawassa water, Ethiopia. International Journal of Water Resources and Environmental Engineering. 2016; 8(4); 52-58.
14. APHA. Standard Methods for the examination of water and waste water American Public Health Association. 1998;874.
15. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's manuel of determinative bacteriology, 9th Edition, Wolters Kluwer Company, New York. 1994; Pp. 87-112.
16. Oranusi SU, Oguoma OI, Agusi E. Microbiological quality assessment of foods sold in student's cafeterias. Global Research Journal of Microbiology, 2004; 3:1-7. 16.
17. Cheesbrough, M. Microbiological test: District Laboratory Practice in Tropical Countries. In: A. Cremer and G. Evan (Eds). Cambridge University Press, UK. 2003; 1-226.
18. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 1987; 4:406-425.
19. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution,* 1985;39:783-791.
20. Jukes TH, Cantor CR. Evolution of protein molecules. In: H. N. Munro, (ed), *Mammalian Protein Metabolism* 1969; 21-132). New York,
21. Bauer AW, Kirby WM, Sherris JC and Truck M. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology 1996; 45(4), 493- 496.
22. National Committee for Clinical Laboratory Standards (NCCLS). *Performance Standards for antimicrobial Susceptibility testing: Sixteenth Informational Supplement.* CLSI document 2006;M100-S111. Pennsylvania, USA.
23. Matyar F, Kaya A, Dincer S. Distribution and antibacterial drug resistance of *Aeromonas* spp. from fresh and brackish waters in Southern Turkey. Annals of Microbiology. 2007;*57* (3):443-447

24. Krumperman PH. Multiple antibiotic indexing of *Escherichia coli* to identify high-risk sources of feacal contamination of foods. Applied Environmental Microbiology. 1985;46: 165-170.
25. Agwaranze DI, Ogodo AC, Nwaneri CB, Agyo B. Bavteriological examination of well water in Wukari, Nigeria. International Journal of Scientific Research in Environmental Sciences. 2017;5(2)., 0042-0046.
26. Anyanwu CU, Okoli EN. Evaluation of the bacteriological and Physico-chemical quality of water supplies in Nsukka, Southeast, Nigeria. African Journal of Biotechnology. 2012;11(48):10868-10873.
27. Olatunji MK, Kolawole TA, Albert BO, Anthony IO. Assessment of water quality in Asa River and its indigenous Clariasgariepinus fish. International Journal of Environmental Research and Public Health. 2011;8, 4332-4352.
28. Shittu BO, Olaitan JO, Amusa TS. Physico-chemical and bacteriological analyses of water used for drinking and swimming purposes in Abeokuta, Nigeria. African Journal of Biomedical Research. 2008;11 (3):285-290.
29. Yahaya AS, Hero MI, Akhter AA. Bacteriological and mycological assessment for water quality of Duhok reservoir. Jordan Journal of Biological Sciences. 2013; 6 (4): 308-315.
30. Romulus AM, Mutemi ME, Kennedy M, Cecilia MM. Physicochemical and bacteriological quality assessment of shallow wells in Kitui town Kenya. Journal of Environmental Science and Water Resource. 2012; 1(2);27-33.
31. Okunye OL, Odeleye FO. Bacteriological investigation of well water samples from selected market locations in Ibadan Nigeria. International Journal of Pharmaceutical Science Invention, 2015;4:32-36.
32. Saif NA, Mohammed IY, Paulser JR, Musharafi SK. Survival and Growth of Antibiotic Resistant Bacteria in treated wasterwater and water distribution system and their implication in Human Health: A Review. The International Arabic Journal of Antimicrobial Agents. 2012; 4(4);31-10.
33. Samantha LL, James, MH. Antimicrobial Reistance, Food Safety, and one's Health: The need for convergence. Annual Review of Food Science and Technology 2016; 7: 287-312.