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Antibiogram of Bacteria Isolated From River Niger within Lokoja Metropolis.

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ABSTRACT

Water samples were collected from five different locations (Jimgbe, Gadumo, Ganaja, Adankolo and Old Market) along River Niger in Lokoja metropolis and analyzed microbiologically and physicochemically using standard methods. Susceptibility of isolated bacteria to commercial antibiotics was also assessed. The results showed that pH(at 25-27^{0}C) ranged from 7.0 to 8.0, temperature ranged from 29 to 31^{o}C, chloride level ranged from 0.32 to 2.04 mg/ml, alkalinity ranged from 8.755 to 8.905 mg/ml, bicarbonate ranged from 1.933 to 2.010 mEq/l, turbidity ranged from 5 to 10 NTU, total solid ranged between 0.075 to 0.321 mg/ml, total suspended solid ranged between 0.002 to 0.180 mg/ml while total dissolved solid ranged between 0.023 to 0.420 mg/ml. The mean total viable count ranged from 2.0 to 4.0 x 10^5 CFU/ml while mean total coliform count ranged from 0 to 8.0 x 10^5CFU/ml. The most probable number (MPN) count ranged from 1600 to ≥ 2400 MPN/100ml. Predominant bacteria isolated included *Staphylococcus aureus*, *Baccilus* sp, *Klebsiella* sp., *Proteus* sp., *Enterobacter aerogenes*, *Eschericia coli Shigella dysenteriae,* and *Salmonella* sp. The antibiogram carried out using the disc diffusion technique showed that *Staphylococcus aureus* was more sensitive to Erythromycin and least to Ampiclox, *Baccilus* sp most sensitive to Streptomycin and Rocephin and least to Ampiclox. *Proteus* sp, *Salmonella* sp, *Enterobacter aerogenes* and *Klebsiella* sp were highly sensitive to all antibiotics, *Eschericia coli* most sensitive to Ciprofloxacin and Gentamycin and resistant to others while *Shigella dysenteriae* were highly sensitive to Tarivid and resistant to Amoxacillin and Streptomycin. The resistance of *Eschericia coli* to 80% of the antibiotics tested is of public health concern.

Keyword: Antibiogram, Physico-chemical Susceptibility, Resistant, River Niger

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INTRODUCTION

Water covers 70.9% of the Earth's surface [1]. And is vital for all forms of life. Only 2.5% of the Earth's water is fresh water, and 98.8% of the water is in ice and ground water. Less than 0.3 of all freshwater is in rivers, lakes and the atmosphere, and an even smaller amount of the Earth's fresh water (0.003%) is contained within biological bodies and manufactured products [2]. Safe drinking water is essential to humans and other life forms. 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 [3].

Quality drinking water is essential for life. Unfortunately, in many countries around the world, water has become a scarce commodity as only a small proportion of the populace has access to treated water [4]. Alternative sources of water such as rain water and ground water have become major sources of drinking water for people living in new settlements and some residents who do not have access to treated water in Nigeria. The need to access the quality of water from some of these alternative sources has become imperative because they have a direct effect on the health of individuals [5]. 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 [6].

The increasing pollution of surface water with domestic and industrial wastes coupled with the alarming cost of construction of water treatment plants and distribution network for human use has made ground water an attractive and important option in the social and economical development of many communities [7]. Many infectious diseases are transmitted by water through the fecal-oral route. Unsanitary water has particularly devastating effects on young children in the developing world. Each year, >2 million persons, mostly children <5 years of age, die of diarrheal disease [8-10].

Although water can contain unwanted chemicals (from natural sources and agricultural activities), the greatest risk to human health is from faecal contamination of water supplies causing water-borne diseases. The consequent illnesses are mostly treated with antibiotics. Unfortunately, there has been development of antimicrobial resistance by many strains of microorganisms which is now making it difficult to treat some infectious diseases. Drug resistant strains have been reported among Staphylococci, Gonococci, Pneumococci, Enterococci and gram negative bacteria including Salmonella, Shigella, Klebsiella, Escherichia coli, Pseudomonas as well as among Mycobacterium tuberculosis [11, 12].

The discovery of antimicrobial agent as a major impact on the rate of survival from infections. However, the changing patterns of antimicrobial resistance caused sulphura demand for new antibacterial agents [13]. The effectiveness of currently available antibiotics is decreasing due to the increasing number of resistant strains causing infections [13, 14]. Drug resistant strains have been reported among staphylococci, gonococci, pneumococci,

enterococci, and gram negative bacteria including *Salmonella*, *Shigella*, *Klebsiella*, *Escherichia coli, Pseudomonas* as well as among Mycobacterium tuberculosis [11, 15].

This study was undertaken to investigate the microbial population and types as well as the antibiotic profile on the isolated organisms along the river Niger in five settlements areas i.e Jimgbe, Gadumo, Ganaja Village, Adankolo and Old market all in Lokoja and Ajaokuta Local Government area, since most of the location used the water for domestic, irrigation, recreation and drinking purpose.

MATERIALS AND METHODS

Study Area and Sample Collection

The study area, Lokoja is situated at 7.8^0 North latitude, 6.74^0 East longitude and 55 meters elevation above the sea level. Lokoja is a town located in the middle-belt of Nigeria, having about 60,579 inhabitants.

River water samples were collected from five different locations in Lokoja which were; Jimgbe, Gadumo, Ganaja, Adankolo and Old-Market (Figure 1). The water samples were collected into sterile bottles assigned to each location during the early hours of the day as described by [16]. The water samples were immediately stored in an ice chest before it was transferred to the laboratory for further analysis.

Standard plate count

The total count was conducted by pour plate technique on plate count agar (PCA) and counting the colonies developed after the incubation at37^OC for 24hours according to [17].

Detection and Enumeration of Coliforms

Enumeration of coliform was carried out according to the method described by [17]; Each tube was inoculated into 5sets of tubes as follows; first, 10ml into tube containing 40ml of lactose broth, usually designated as double strength lactose broth (DSLB) with Durham tubes, then 1.0ml of the 20ml of lactose broth, usually designated as single strength lactose broth (SSLB) with Durham tubes and then 0.1ml inoculated into five tubes each containing 20ml of lactose broth usually designated as single strength lactose broth (SSLB) with Durham tubes. The tubes were incubated at 35^OC for 24- 48 hours. Following incubation, tubes showing gas production were counted and compared with MPN table adapted from [17], for determination of most probable number (MPN) of coliform per 100ml of water.

The media used for bacteriological analysis of water include plate count agar (PCA) nutrient agar (NA), Lactose broth (LB) and Eosin Methylene blue agar (EMB). All the media used were weighed out and prepare according to the manufacture's specification, with respect to the given instruction and directions. A serial dilution method was for viable count and the

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presumptive test for coliforms. The sterility of each batch of test medium was confirmed by inoculating one or two uninnoculated tube or plates along with the inoculated tests. The uninoculated tube or plates were examined to shown no evidence of bacterial growth was discarded. The pure cultures of bacterial isolates were subjected to various morphological and biochemical characterization tests to determine the identity of the bacteria isolates with reference to Bergey's Manual of Determinative Bacteriology.

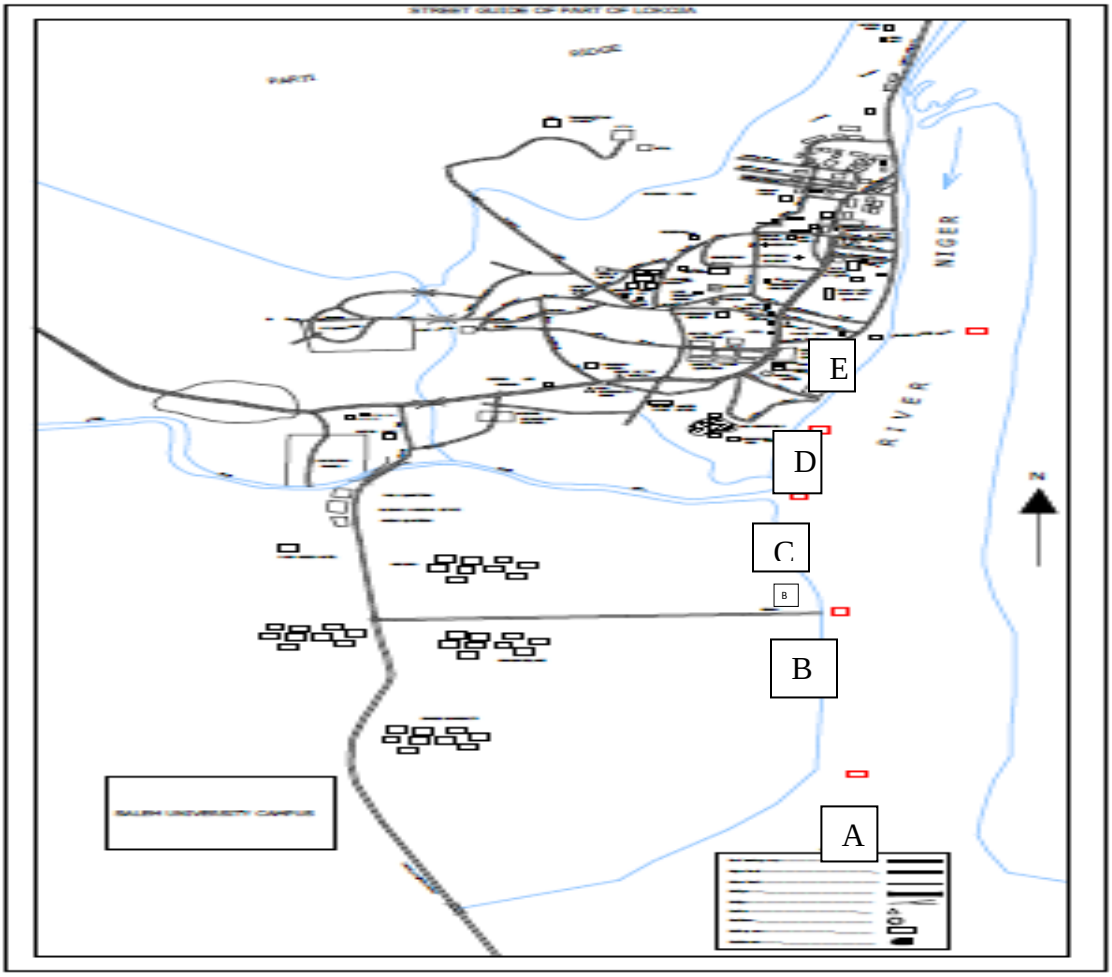

Figure 1: Map of the sampling locations.

KEY: A= Jimgbe B= Ganaja Village C= Gadumo D= Adankolo E= Old Market
——— = Dual road ○ = Banks ● = Settlement □ = Build up area ═ =Major road ╟╢ = Bridge

Pysicochemical Analysis

The pH of the water samples was measured by using the electrometric methods using a pH meter (model: HP 2211 ph/ORP meter) and other physicochemical parameters such as Total dissolved solids (TDS), Total Suspended Solid (TSS) **,** Total Solid (TS), Total Alkalinity, Chloride, Bicarbonate and Turbidity were analysed by standard methods as described by the American Public Health Association [17].

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Sensitivity Test

The antibiotics susceptibility of the isolate was determined by the disc diffusion method on Muellar Hinton Agar. The antibiotic multi-disc; Made in Nigeria by Maxicare Medical Laboratory, containing for Gram negative (G-ve); septrin, Chloranphenicol, sparfloxacin, ciprofloxacin, amoxicillin, augmentin, gentamycin, pefloxacin, tarivid, streptomycin and for Gram positive (G +ve); pefloxacin, gentamycin, ampiclox, zinnacef, amoxicillin, rocephin, ciprofloxacin, streptomycin, septrin, erythromycin, was used. The inoculum was standardized by adjusting it's density to equal of a barium sulphate ($BaS0_4$) at 0.5 McFarland turbidity standards, and incubated at 37^{o}C for 18hrs. The diameter of the zone of clearance was measured to the nearest millimeter (mm) [15].

RESULTS

The descriptions of the sampling site (Jimgbe, Gadumo, Ganaja Village, Adankolo and Old Market) are shown in (Table 1). About 100% of the River water in the different location have activities (swimming, bathing, urine waste, feacal discharge, fishing, irrigation, abattoir waste disposal, dumping of refuse etc.) carried out around them. And most of the water bodies are close to settlement and farm land.

The pH values range from 7.0 to 8.0 for the analyzed samples (Table 2). The colour and odour of the river water in Adankolo and Old Market were brownish and offencive, while for Jimgbe, Gadumo and Ganaja village was Clear and Odourless. The temperature ranged between 29.0 and 31.0 ^{o}C , the chloride level ranged between 0.04 to 2.04 mg/ml. The Bicarbonate level ranged between 1.933 to 2.010 mEq/l. The turbidity level ranged between 5 and 10 NTU. The total solid (TS) ranged between 0.036 to 0.321 mg/l, the total suspended solid (TSS) ranged between 0.002 and 0.180 mg/l and the total dissolved solid (TDS) ranged between 0.023 and 0.420 mg/l.

The highest mean total viable count at 37^{o}C was 4.0 cfu/ml x 10^5 for Old Market and the least was 2.0 cfu/ml x10^5 for Jimgbe and Ganaja, while the highest mean total coliform count was 3.0 cfu/ml x10^5 for Gadumo and the least with no coliform count at 10^5 dilution factor was Jimgbe. (Table 3).

A total of eight bacterial species were identified. These were *Salmonella sp*., *Klebsiella sp., Staphylococcus sp., Proteus sp., Enterobacter aerogenes, Escherichia coli, Shigella dysenteriae and Bacillus sp*.

Table 4: shows the most probable number of coliforms in the water sample from the various locations. The most probable number (MPN) index per 100ml for Jimgbe was 1,600 while for Gadumo, Ganaja Village, Adankolo and Old market was ≥ 2,400.

Table 5 shows the antibiogram parterns of *Staphylococcus aureus* and *Bacillus.* Sp. to Gram positive (G +ve) antibiotics discs, while Table 6 shows the antibiogram for gram Negative

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organisms; *Salmonella* Sp., *Klebsiella* Sp., *Proteus* Sp., *Enterobacter aerogenes*, *Shigella dysenteriae* and *Eschericia coli*.

Table 1: Description of sampling site of the water samples.

Location	Nature of the river	Activities carried out around the river body
Old Market (OM)	Large body of water and light brown in colour.	Selling and buying, Abatoir is present, Fishing, Individuals bathing around the river body, Washing, urinating, stooling and dumping of refuse around the river body.
Adankolo (A)	Close to the bridge along the road.	Dumping of refuse, swimming and relaxation.
Ganaja village (Gj)	Close to the road. Wide and deep with a large flow of water.	Transporting of goods and people with ferry, fishing and boat ride.
Gadumo (Gd)	Close to settlement and farmland,	Boat ride, Farming, swimming, relaxation and fishing.
Jimgbe (J)	Very far from settlement, along forest path.	Bathing, swimming, irrigation, farming, building, washing and block molding.

Table 2: Physiochemical analysis of River Niger water in Lokoja

Parameters	Standard (WHO/ SON)	Water samples				
		Old market	Adankolo	Ganaja	Gadumo	Jimgbe
Colour	Clear	Brownish	Brownish	Clear	Clear	Clear
Odour	Odourless	Offensive	Odourless	Odourless	Odourless	Odourless
pH (mg/l)	6.5 - 8.5	7.0	8.0	7.5	7.0	7.6
Temperature (^{0}C)	-	30.0	31.0	31.0	29.0	29.0
Chloride (mg/ml)	200 mg/ml	0.05	2.04	0.32	0.04	0.04
Alkalinity (mg/l)	100 mg/l	8.755	8.845	8.760	8.800	8.905
Bicarbonate (mEq/l)	100 mEq/l	1.936	1.933	1.938	1.956	2.010
Turbidity (NTU)	1-10 NTU	10	10	5	5	5
Total Solid (TSL;mg/l)	< 500mg/l	0.134	0.321	0.083	0.075	0.036
Total Suspended Solid (TSS; mg/l)	40 – 80 mg/l	0.092	0.180	0.060	0.005	0.002
Total Dissolved Solid (TDS;mg/l)	< 300mg/l	0.420	0.141	0.023	0.070	0.034

Table 3: Total viable bacterial and coliform counts of the water samples from River Niger Lokoja.

Water samples	Mean total bacterial count (cfu/ml x10^5)	Mean total coliform count (cfu/ml x 10^5)
Old market (OM)	4.0	3.0
Adankolo (A)	3.0	1.0
Gadumo (Gd)	3.0	8.0
Ganaja (Gj)	2.0	1.0
Jimgbe (J)	2.0	0.0

Note: Values are means of replicates.

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Table 4: Most probable number (MPN) of coliforms in the water sample from various locations.

Water samples	No of tubes giving positive reactions			MPN index per 100ml
	5 of 10ml each	5 of 1ml each	5 of 0.1ml each	
Old market (OM)	5	5	5	≥2,400
Adankolo (A)	5	5	5	≥2,400
Ganaja (Gj)	5	5	5	≥2,400
Gadumo (Gd)	5	5	5	≥2,400
Jimgbe (J)	5	5	4	1,600

Table 5: Antibiotic sensitivity Test (Gram positive; G+ve)

Antibiotics	Concentration	Zones of inhibition (mm)	
		Staphylococcus aureus	***Bacillus* sp.**
APX-Ampiclox	30ug	11.0	0.0
Z-Zinnacef	20ug	19.0	24.0
AM-Amoxacillin	30ug	18.0	24.0
R-Rocephin	25ug	25.0	28.0
CPX-Ciprofloxacin	10ug	25.0	26.0
S-Streptomycin	30ug	19.0	28.0
SXT-Septrin	30ug	20.0	24.0
E-Erythromycin	10ug	26.0	23.0

Note: Values are means of replicates.

Table 6: Antibiotic Sensitivity Test (Gram negative ; G-ve)

Antibiotics	Concentration	Zones of inhibition (mm)					
		***Salmonella.* Sp**	***Proteus.* sp.**	***E. aerogenes***	***Klebsiella* sp.**	***Escherichia coli***	***Shigella dysenteriae***
SXT-Septrin	10ug	23.0	20.0	20.0	26.0	0.0	10.0
CH-Chloramphenicole	10ug	28.0	28.0	11.0	28.0	0.0	10.0
SP-Sparfloxacin	30ug	24.0	28.0	28.0	28.0	0.0	24.0
CPX-Ciprofloxacin	20ug	28.0	27.0	28.0	28.0	20.0	29.0
AM-Amoxacillin	30ug	23.0	10.0	11.0	10.0	0.0	0.0
AU-Augmentin	25ug	23.0	11.0	10.0	11.0	0.0	22.0
CN- Gentamycin	10ug	28.0	24.0	23.0	24.0	29.0	27.0
PEF-Pefloxacin	30ug	28.0	26.0	28.0	27.0	0.0	26.0
OFX-Tarivid	30ug	26.0	28.0	29.0	28.0	0.0	28.0
S-Strptomycin	10ug	26.0	12.0	11.0	11.0	0.0	0.0

Note: Values are mean of replicates.

DISCUSSION

From the results obtained, it was observed that the pH values show acceptable limits for water (6.5-8.5), and the temperature is warmer within the water (25-27^{o}C) which is within the range that bacteria proliferates as reported by[18]. Most bacteria are neutrophils and tend to

survive well in a neutral pH range. The variation in the pH of water samples may be due to the presence of varying amount and types of mineral matter [19]. Temperature is the most salient factor influencing microbial growth in drinking water [19]. The study showed the range of the temperature values obtained to be between 29 to 33.9^{o} C which is suitable for the proliferation of mesophiles. [20] reported that most of the bacteria isolated from water are mesophiles.

The total solids (TS), Total suspended solids (TSS) and Total dissolved solids (TDS) in the different river water samples is in accordance to the accepted standard (< 500mg/l, 40 – 80 mg/l and < 300) which may be due to self purification of the water bodies. The chloride level in the water falls within the range (< 10 mg/l), and this chloride present in the water affects the pH level of the water hence making the river water to maintain the optimum temperature for bacteria proliferation. The turbidity level of the different water samples is normal for potable water (1-10 NTU). The alkalinity falls within the accepted limit for the different water samples (> 7) . And the Bicarbonate level of the different water samples was bellow the accepted level of Bicarbonate (22-26 mEq/l) that should be present in potable water, which also renders the river water unfit for consumption. The bicarbonate level of the different wate samples fall between the range of 1.933 – 2.010 (mEq/l).

The presence of *Eschericia coli* in water samples is an indication of fecal coliform contamination of the water, and also *Eschericia coli* normally lives in the human large intestine and causes no harm, a few strains can cause serious illness including severe diarrhea and kidney failure, *Staphylococcus* is a normal floral, *Salmonella*, *Enterobacter aerogenes* and *proteus* inhabits the intestine of man and animal and in multiple environmental habitat, including long-term health care facilities and habitats, *Klebsiella* sp. Frequently cause human nosocomial infection, it accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemias, and soft tissue infections, *Shigella dysenteriae* causes shigellosis and Bacillus species are important in the natural or artificial degradation of waste products. The high total viable bacterial and coliform count from different samples locations indicated presence of bacteria and coliforms which is a sure indication of the pathogenicity of the organisms present in the water bodies, and since coliforms must be present where other pathogens are present, their presence in the water indicates the presence of pathogenic micro organisms.

The most probable number (MPN) of the water samples is another clear indication that the river water is contaminated in the different locations in lokoja, since the value was > 10 per 100ml of each samples. As coliform bacterial are very abundant in human wastes, they are much easier to locate and identify in feacal polluted water than other pathogens that cause severe diseases. The resistance of *Eschericia coli* to majority of antibiotics used is of great health concern. The variation in susceptibility and resistance of the isolates to different antibiotics could be attributed to the difference in the concentration of antibiotics, source of isolate and drug resistance transfer [21].

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CONCLUSION

Evidence provide in this study indicated that high levels of antibiotic resistance were observed within the water body, this may be due to the presence of environmental pollutants in the river. The people leaving in the study area are at risk since the work have shown that there is contamination of water used as source of domestic , irrigation, recreation and drinking water in that area are contaminated by fecal coliform. Regulatory agencies should intensive their effort toward providing clean and portable water to the public and also engage in a campaign for cleanliness of the environment, good refuge and sewage disposal systems since their potential impact on the ecosystem may be much greater than its effect on human life.

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