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Antibiotics Susceptibility Pattern of Bacterial Isolates Obtained from Potable Water Sources in Okerenkoko Community, Delta State, Nigeria

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Abstract

Safety and purity of drinking and cooking water sources in rural and riverine communities like Okerenkoko have posed a challenge for scientific discuss in recent times. Water devoid of both chemical and microbiological contaminants have been identified as a major key to wellness of any populace. In this study, eight potable water sources were obtained and evaluated for the study; the potable water sources obtained were treated to a 10-fold serial dilution and plated for aerobic bacterial, fungal, *Salmonella-Shigella* and *Escherichia coli* count. The antibiotics sensitivity testing employed the modified Kirby- Bauer method using the Abtek biological multiple disc. The multiple drug resistance was mathematically deduced using the standards and breakpoints. The NMU and George borehole had 5.8 Log_{10}CFU/ml and 5.4 Log_{10}CFU/ml for the total aerobic bacterial count while the total fungal count were 3.1 Log_{10}CFU/ml and 3.6 Log_{10}CFU/ml respectively. The culturable flora observed during the study were *Bacillus* sp, *Escherichia* sp., *Staphylococcus* sp., *Streptococcus* sp., *Shigella* sp., *Proteus* sp., *Pseudomonas* sp., *Klebsiella* sp., *Vibrio* sp. *Micrococcus* sp. and *Escherichia coli*. The percentage resistance for the antibiotics revealed cefuroxime and ceftazidime had 64%, while nitrofurantoin had 71% and ofloxacin had 36%. *Proteus* sp. (1) had an MDR of 1.0, *Klebsiella* sp. (3) had 0.67 while *Salmonella* sp. and *Escherichia coli* had a MDR of 0.22 and 0.11. The findings of the study underscores the health challenges associated with the usage of the available potable water sources in Okerenkoko community. There is need for a community-wide campaign and sensitization on some basic water purification approaches as possible corrective actions to improve the quality of water; government must intervene and provide a routine and robust evaluation for both water safety and sanitary quality in rural communities.

Keywords: *Antibiotics, Susceptibility, Potable, Multi-Drug Resistance, Riverine, Kirby-Bauer*
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I. INTRODUCTION

Water is an important factor for living things to survive; as it is widely said to be a universal solvent. It is considered crucial for physiological functions and has been identified over the years to play a number of religious, social and economic roles in society (Ovonramwen *et al*., 2020). Other roles played by water are agricultural, domestic, industrial and environmental. Safe-to-drink and cook water are said to be potable water. They are considered fit and wholesome for a vast number of domestic activities because it is believed to have a low concentration of microbes. Water in its purest form may be said to be potable when it is free of any contamination from any form of exogenous biological or chemical agents. In recent times, water sources have largely been tainted by a number of anthropogenic forces. Water may be classified either as surface or groundwater. Groundwater is the category of water that is trapped below the earth crust in the form of borehole water. According to Asionye *et al*. (2023) underground water is the most frequency accessed source of healthy water. In most rural and riverine communities in the Niger Delta region of Nigeria; Surface water still accounts for the most accessed source of quality drinking water especially in the areas where the government may not have adequate cover. Most of these surface water may have been influenced through its tributaries and may

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receive contaminants because they become receptacles for the industries within the communities.

Poor sanitary practices in and around the water bodies have also contributed to the impact on the available potable water sources. Some other reports have identified the cost of the packaged water to have worsened the demand on the locally available options in the community. Many riverine and coastal communities have challenges of accessing adequate potable water sources.

II. Globally, there exist an invasive prevalence of antimicrobial resistance of organisms of health importance in the world today. In so many countries today, the trend at which antibiotics resistance has surged is increasingly worrisome (Haile *et al.,* 2022). The spike in the resistance of the antibiotics have been hinged on the loss of efficacy of therapeutic and curative agents; due to the misuse and abuse of drugs (Javeed *et al*., 2011). The antibiotics susceptibility pattern of the bacterial isolates has continually worsened leading to a wide array of multiple drug resistance (MDR). Efflux mechanism have been identified as a common resistance mechanism used by microbes to modify some target antibiotics. The number of cases of multiple drug resistance pattern of bacterial isolates have not been accounted in some peer reviewed investigations; this is because there has been a poor documentation for a number environmental media to serve as a reference for further studies, especially for the flora of the aquatic ecosystem. The worsening trends have necessitated the implantation of a compulsory laboratory test prior to the administration of drugs. Others have also attributed the role of abuse and misuse in the application of antibiotics as a major source of infiltration of these resistant species. The antibiotic resistance pattern of enteric pathogens from commonly consumed water has been identified as a major issue for rural dwellers in Nigeria. This study targets to evaluate the antibiotic susceptibility pattern of the bacterial isolates obtained from the potable water sources in the Okerenkoko community, Delta State, Nigeria.

III. Materials And Methods

A. Study Area

Okerenkoko, Gbaramatu is situated in Warri South-West in Delta State, Nigeria. Their language is called Ijaw and they are freely called Ijaw people. They are the home to the famous Escravos River which is one of the popular hubs for oil and gas operations, shipping and other recreational activities in the Niger Delta. Okerenkoko community, in Gbaramatu kingdom is located in Warri South-West Local Government Area, of Delta State, Nigeria. They are commonly referred to as the Ijaw speaking “Ijaw people” area in the Escravos River is predominantly into fishing and aquaculture. Okerenkoko community is host to a number of Crude oil Tank Farm and Terminals, operated by major oil companies. The activities both religious and cultural in the Okerenkoko community enjoys the serenity of the Atlantic Ocean, through the Escravos. The major sources of water in the community are boreholes and surface water. Most of the resources have been impacted either directly or indirectly.

B. Data collection

A total of eight (8) potable water sources were aseptically obtained, labelled and transported in the ice chest to the Springfield Laboratory Choba, Rivers State. The different locations in Okerenkoko community were geo-referenced and Geo-mapped showing the sampling points as presented in Figure 1.

C. Microbiological Evaluation of Potable Water Samples

1. *Determination of Total Aerobic Bacterial Count (TABC)*

The total aerobic bacterial count was measured by spread-plate approach on plate count agar (Oxoid, United Kingdom) was used. The water samples were diluted serially using a 10-fold serial dilution. Aliquot of the diluted samples. The cultured plates were placed in the incubator for a day at 37^{o}C. Colonies of bacteria isolates were counted and represented in CFU/ml (Asionye *et al*., 2023).

2. Determination of Total Fungal Count (TFC).

The fungal counts of the potable water samples were determined by spread plating on the potato dextrose agar. Total heterotrophic bacterial counts for the different potable water sources were determined using the spread plate method on nutrient agar (Oxoid, U K). Ten-fold serial (10-fold) using sterile distilled water as a diluent. Cultured plates were counted for visible colonies and presented in CFU/ml (Effiong *et al*., 2020).

3. Determination of Total Salmonella–Shigella counts in water.

The potable water samples were evaluated for the presence of *Salmonella–Shigella* to ascertain the presence of enteric pathogens using the *Salmonella* and *Shigella* sp. The potable water samples were diluted serially and plated on the solidified agar (Nwachukwu *et al*., 2010). The plates were incubated at 37^{o}C for 24hrs and the characteristic colonies were counted for either of the isolates. Mathematically represented

$$Titre = \frac{\text{Average Number of colonies x Dilution factor}}{\text{volume plated}}$$

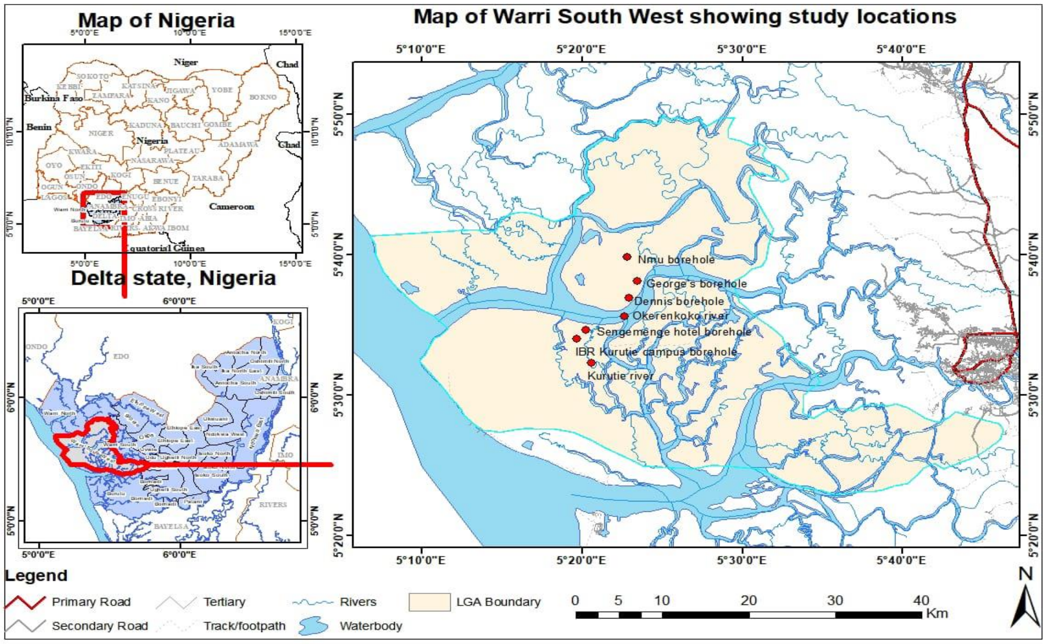

Figure 1. Map of Warri South-West showing the study locations.

4. Determination of Escherichia coli counts in water

The potable water samples were evaluated for the presence of *E. coli* as an indicator organism (Orji *et al*. 2015). The *Escherichia coli* counts of the potable water sources obtained from Okerenkoko was determined using the spread plate method on eosin methylene blue media. The determination of the *E. coli* in the potable water samples on the EMB medium followed the same approach.

5. Determination of antibiotics susceptibility of bacterial isolates

Antibiotics susceptibility of the bacterial isolates obtained from the potable water samples were ascertained using a Modified Kirby-Bauer multiple disc method and the breakpoints were compared using the Clinical Laboratory Standard Institute manual. The Composition of the multiple disc obtained from Abtek Biological Limited; the Gram Negative Disc Ceftazidime (CAZ-30µg), Cefuroxime (CRX-30 µg), Gentamicin (GEN-10 µg), Cefixime (CXM-5 µg), Ofloxacin (OFL-5 µg), Augmentin (AUG-30 µg), Nitrofurantoin (NIT-300 µg), Ciprofloxacin (CPR-5 µg) while the Gram-positive had discs had contained Ceftriaxone (CTR-30 µg), Cloxacillin (CXC-5 µg). The zones of inhibition were measured after a 24 hrs exposure to the disc and Kirby-Bauer approach. The diameters recorded were compared against the CLSI breakpoint (Trojan *et al*., 2016).

IV. Results

The microbial quality of the potable water sources was presented in Figure 3. Total aerobic bacterial count in the water sample S1OK and SIKR had the maximum microbial concentration of about 5.8 Log_{10}CFU/ml and 5.4 Log_{10}CFU/ml while the total fungal count were 3.1 Log_{10}CFU/ml and 3.6 Log_{10}CFU/ml respectively. The aerobic bacterial count for the community borehole S5OK and control (Flourish sachet water) samples had 3. Log_{10}CFU/ml and 3.3 Log_{10}CFU/ml. The borehole water (S1OK) and surface water (S3OK) had a high concentration of the Total *Escherichia coli* count of about 3.6 Log_{10}CFU/ml and 4.5 Log_{10}CFU/ml.

Table 1 shows the confirmatory study from the Selenite -F enrichment test, suggesting the S3Ok had a higher degree of the *Salmonella* sp.

Table 2 shows the diameter of zones of inhibition of the antibiotics against the bacterial isolates. The *Proteus* sp. (1) had 10.0mm for gentamicin, nitrofurantoin had 12.4 mm, ofloxacin had 10.0 mm while ceftazidime had 8.4 mm. *Streptococcus* sp. (6) had 30.0 mm and 18.0 mm for ofloxacin and gentamicin respectively.

Table 3 shows the multi-drug resistance profile of the bacterial isolates, *Proteus* sp. was observed to have a MDR of 1.0, *Klebsiella* sp. (3) had an MDR of 0.67, while *Staphylococcus* sp. (7) was 0.89. *Micrococcus* sp. (5) had 0.67. *Shigella* sp (14) had MDR of 0.79 while *Vibrio* sp. had 0.89.

The antibacterial susceptibility pattern of the bacterial isolates was presented in Figure 2. 12 isolates were sensitive to gentamycin, nine (9) isolates were susceptible to ofloxacin while 7 were susceptible to cefuroxime. augmentin for 12 isolates showing resistance to the antibiotics, erythromycin for 11 and cefixime was 10, both cefuroxime and ceftazidime both had 9 resistant cases.

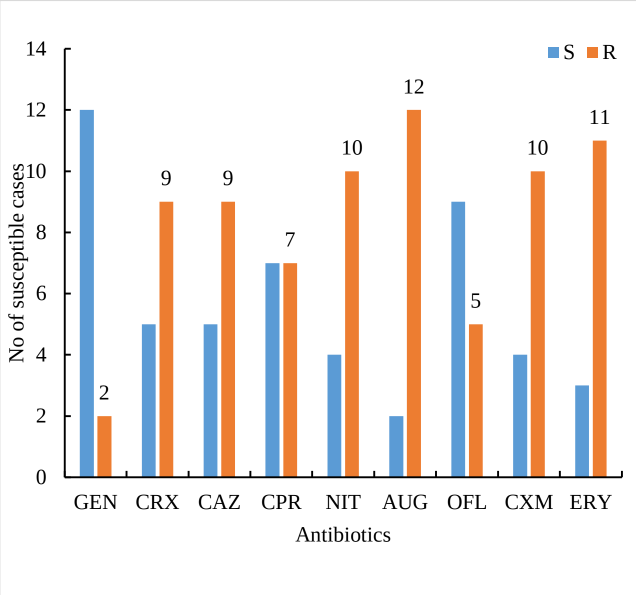

Figure 2. Antibiotics susceptibility pattern of the bacterial isolates

Table 1. Selenite enrichment and parasitic assessment report.

Samples	*Salmonella* and *Shigella* sp.	Acetone-wet mount
S_1k	+	-
S_2k	+	-
S_3K	+	-
S_1O	+	-
S_2O	+	-
S_3O	+++	
S_4O	+	-
S_5O	+	-
FL	+	-
CO	+	-

Key: S1KR=NMU/Krutie community borehole, S2KR=Sengemenge Hotel borehole, S3kR=Krutie Community River , S1OK= George's borehole, S2OK=NMU Okerenkoko borehole, S4OK=Okerenkoko Community borehole, S5OK=Dennis borehole, FL= Flourish Sachet water(Control), CO= Concept Sachet.

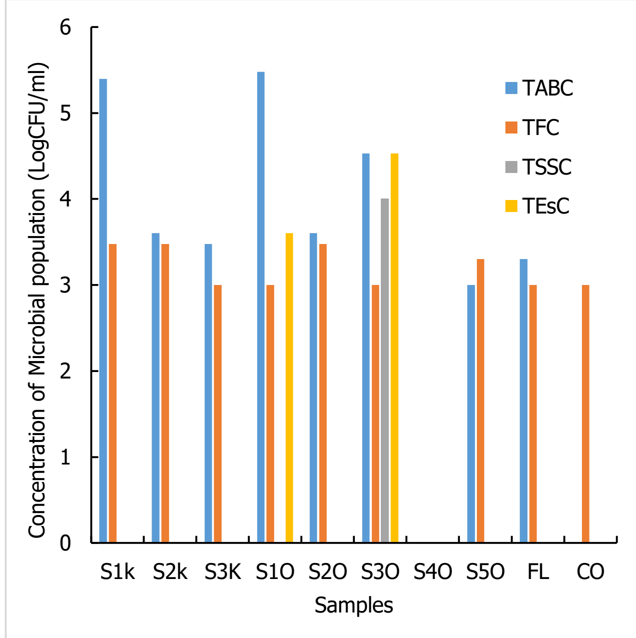

Figure 3. Microbial quality of portable water sourcesobtained in Okerenkoko. Key: S1KR=NMU/Krutie community borehole, S2KR=Sengemenge Hotel borehole, S3kR=Krutie Community River, S1OK= George's borehole, S2OK=NMU Okerenkoko borehole, S4OK=Okerenkoko Community borehole, S5OK=Dennis borehole , FL= Flourish Sachet water(Control), CO= Concept Sachet.

V. DISCUSSIONS

The report obtained from the study underscored the significant microbial quality in the potable water accessed by the populace in Okerenkoko. The samples of the potable water had a range of total aerobic bacterial count ranging from 5.4 to 5.8 Log_{10}CFU/ml. The water sample S1OK and SIKR concentration values of 5.8 Log_{10}CFU/ml and 5.4 Log_{10}CFU/ml respectively; total fungal count recorded were 3.1 Log_{10}CFU/ml and 3.6 Log_{10}CFU/ml respectively. The aerobic bacterial count for the community borehole S5OK. Our results were in agreement with the work of Nnadozie (2016) whose investigation identified the presence of enteric pathogens in drinking water sources in Port Harcourt metropolis Rivers State. Poor sanitary conditions and disposal of wastewater into the receiving surface water have also been identified to have increased the surge in the presence of pathogens in potable water sources. Although this does not rule out or limit the activities of seepages and runoff as a pivotal cause of most pollution of surface water (Sasakova *et al*., 2018).

Table 2: Antibiotics susceptibility pattern of bacterial isolates

Isolates	GEN	CRX	CAZ	CPR	NIT	AUG	OFL	CXM	ERY	Tentative Identity
1	10.0	0.0	8.4	0.0	12.4	0.0	10.0	0.0	0.0	*Proteus* sp.
2	21.0	19.0	18.0	25.0	25.0	12.0	22.0	24.0	0.0	*Proteus* sp.
3	21.0	0.0	0.0	22.0	24.0	0.0	25.0	0.0	0.0	*Klebsiella* sp.
4	12.0	0.0	0.0	25.0	0.0	0.0	30.0	0.0	0.0	*Pseudomonas* sp.
5	21.0	19.0	18.0	0.0	0.0	0.0	0.0	0.0	0.0	*Micrococcus* sp.
6	18.0	0.0	0.0	0.0	0.0	0.0	30.0	0.0	5.0	*Streptococcus* sp.
7	22.0	11.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	*Staphylococcus* sp.
8	20.0	0.0	15.0	19.0	27.0	0.0	26.0	29.0	0.0	*Bacillus* sp.
9	11.0	0.0	0.0	5.0	0.0	10.0	0.0	0.0	0.0	*Vibrio* sp.
10	15.0	0.0	0.0	0.0	0.0	0.0	29.0	0.0	15.0	*Micrococcus* sp.
11	20.0	22.0	19.0	22.0	30.0	13.0	22.0	20.0	0.0	*Escherichia coli*
12	14.0	0.0	0.0	0.0	0.0	0.0	21.0	0.0	10.0	*Bacillus* sp.
13	16.0	14.0	20.0	20.0	22.0	15.0	17.0	21.0	0.0	*Salmonella* sp.
14	0.0	0.0	0.0	20.0	28.0	0.0	0.0	0.0	0.0	*Shigella* sp.

Table 3: Multidrug Resistance Profile pattern of bacterial isolates

Isolates	GEN	CRX	CAZ	CPR	NIT	AUG	OFL	CXM	ERY	MDR	Tentative Identity
1	R	R	R	R	R	R	R	R	R	1.0	*Proteus* sp.
2	S	S	S	S	S	R	S	S	R	0.33	*Proteus* sp.
3	S	R	R	S	R	R	S	R	R	0.67	*Klebsiella* sp.
4	S	R	R	S	R	R	S	R	R	0.67	*Pseudomonas* sp.
5	S	S	S	R	R	R	R	R	R	0.67	*Micrococcus* sp
6	S	R	R	R	R	R	S	R	S	0.67	*Streptococcus* sp.
7	S	R	R	R	R	R	R	R	R	0.89	*Staphylococcus* sp.
8	S	R	S	S	R	R	S	S	R	0.44	*Bacillus* sp.
9	S	R	R	R	R	R	R	R	R	0.89	*Vibrio* sp.
10	S	R	R	R	R	R	S	R	S	0.67	*Micrococcus* sp.
11	S	S	S	S	S	S	S	S	R	0.11	*Escherichia coli*
12	S	R	R	R	R	R	S	R	S	0.67	*Bacillus* sp.
13	S	R	S	S	S	S	S	S	R	0.22	*Salmonella* sp.
14	R	R	R	S	S	R	R	R	R	0.79	*Shigella* sp.
	14	64	64	50	71	87	36.3	72			% Resistance

The *Salmonella Shigella* count for S3O; was 4.0 Log_{10}CFU/ml. these corroborated the earlier report of Megchún-García *et al*. (2018) where they observed a coliform concentration value that surpassed the drinking water sources by almost 10-times as a result of seepage from wastewater from an agro-allied service and septic tank in Mexico.

Microbes associated with the potable water samples were mainly enteric pathogens especially *Escherichia coli* which is an indicator organism of fecal origin. Most of these enteric pathogens may cause severe and life-threatening infections in both adults and children. Diseases such as Salmonellosis, Shigellosis and Vibriosis have been identified in some tropical countries as a highly devastating *Vibrio* species could lead to severe intestinal infections. One of the common diseases ravaging the Okerenkoko community is a form of Salmonellosis. The severity of this infection has been linked to reduce the student work hour and down time due to loss in the nature of the illness associated with the infection. A study by Eboh *et al.* (2017) also reported a wide array of enteric and a number of bacterial identified as nosocomial-related agent from borehole. Furthermore, Onyango *et al*. (2018) identified and isolated *Clostridium* sp from borehole in Kenya. Ordinoha (2011) reported the failure of the workmen that are involved in the drilling process to be as a major cause of the pathogens in the groundwater. The specification of the borehole has been a major contributor to the infiltration and denting of the groundwater. According to Abubakar (2010) fifty percent of the persons living in the rural communities have been identified are exposed to waterborne disease and the percentage could be worse for the riverine communities. The impact of poorly treated and disposal of untreated from a number of activities on the aquatic ecosystem has also contributed to the trend in infections (Owamah *et al*., 2013; Ehiowemwenguan *et al*., 2014; Okereke *et al*., 2014; Adogo *et al*., 2015; Adebawore *et al*., 2016; Sojobi, 2016 and Owamah, 2020). The dominance of moulds of a variety of fungus was identified during the study but was not limited to the *Penicillium* sp. and *Microsporium* sp. These fungal isolates have been identified to correlate to a number of soil fungi and most of them have a severe effect on humans. Similarly, Eboh *et al*. (2017) isolated *Rhizopus* and *Penicillium* sp. from ground and surface water.

The antimicrobial susceptibility of the bacterial isolates as presented in Table 8.0 suggested that *Proteus* sp. denoted as Isolate 1 had an MDR index of 1.0. The report obtained for the isolates *Klebsiella* sp. had a 0.67 MDR index while *Staphylococcus* sp. and *Vibrio* sp. had an MDR of 0.89 while *Salmonella* sp. and *Escherichia* sp. had MDR 0.22 and 0.11 respectively. The *Bacillus* sp. was observed to have an MDR of 0.67. These findings corroborate the report of Odonkor and Addo (2018) for which their investigation recorded a high level of resistance to antibiotics such as Cefuroxime, Nitrofurantoin, Tetracycline and Penicillin with MDR of >2.0. Furthermore, Balasa *et al*. (2021) reported the presence of bacterial isolates with high MDR indices with their report identifying over 43% of the isolates being resistant to four or more antibiotics. In a related study, Ramirez- Castillo *et al*. (2013) identified a wide array of antibiotic-resistant bacteria in water with more than 50% resistance to conventional antibiotics. Water is an oligotrophic medium and may rarely serve as a medium for the cultivation of microbes; upon impact by inorganic pollutants especially on the surface or underground water may induce microbes to device adaptive mechanisms which may increase their rate of resistance. This is evident in the resistance pattern in most of the microbes under study in this present investigation as a number of the microbes were observed to resist up to 80 to 100% of the conventional antibiotics that were tested in the study. *Pseudomonas* sp. with a high MDR can pose a threat of being a secondary pathogen of health importance (Shar *et al*., 2010). The report of Ewelike *et al*. (2022) suggested that the microbes obtained from the portable water sources in Owerri municipal had similar organisms as observed in the present study.

VI. CONCLUSIONS

VII. This study has understudied the microbial quality of the potable water sources in Okerenkoko, Delta State, Nigeria. The presence of enteric pathogens and coliforms were isolated and identified using a battery of biochemical test. The antibiotics susceptibility pattern as presented in the antibiogram typifies the spate of multi-drug resistance pattern for both first and second-line antibiotics including ciprofloxacin and ceftriaxone. This indicates that there might be a spike in the evolution in the drug idiosyncrasy in the Okerenkoko community as it will affect both persons of the low and high socio-economic class. The challenge of the resistance pattern may also worsen if the risk factors are not properly handled and corrected; abuse and misuse of antibiotics may incur more cost on the populace which includes the students of the Nigerian Maritime University, Okerenkoko. There is need for a community and state-wide campaign for awareness on improving the sanitary activities and need to mitigate the pollution phenomena by the government regulatory activities.

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