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Emerging Drug Resistant *Escherichia coli* and *Salmonella* spp. Isolated from Selected Streams in Gboko Town, Benue State

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Abstract

Background: Development of resistance by bacteria is a major problem in antibiotic therapy which has posed a significant challenge to pharmaceutical practitioners and health authorities over time. Widespread use of antibiotics by humans, indiscriminate medical waste disposal are among other factors that have accounted for resistant bacteria in aquatic environment. The aim of the study is to determine the antibiotic susceptibility profile of *E. coli* and *Salmonella* spp. isolated from stream water in Gboko.

Results: Antibiotic susceptibility test on *Escherichia coli* isolates showed highest resistance to Ampicillin 2.6 (82.7%), followed by Gentamycin, 3.7 (76%), Ciprofloxacin (CEP), Nalidixic acid (NA), Augmentin (AU), and Streptomycin (S), had 4 (75%) respectively, while Tarvid (OFX), Pefloxacin (PEF), Septrin (SXT), had 6.6 (63.5%) and the highest susceptibility value is Ceporex (CPX) 7 (57.2%). Further antibiotic susceptibility profile of *Salmonella* spp. isolated from selected stream water in Gboko town, Tarvid (OFX), had the highest of 3 (77.9%), Augmentin (AU) 3.3 (75.5%), Gentamycin (CN) 4 (69.3%), Streptomacin (SXT) 5.6 (57.9%), Pefloxacin (PEF) and Streptomycin (SXT) had 6.6 (40.2%), while the lowest is Nalidixic acid (NA) 7 (37.1%).

Conclusion: Stream waters in Gboko town are contaminated with multi-drug resistant *Escherichia coli* and *Salmonella* spp. which presents a major threat to public health since the waters are commonly used for domestic activities without treatment. The use of stream waters in this area without treatment exposes the inhabitants to pathogenic bacteria which can cause severe diarrhea and other enteric infections. This may be the reason for most of the diarrhea infections experienced by most of the inhabitants of the town, since water from streams are used for irrigation of vegetable farms around the town, swimming and other domestic purposes. Stream water sources from the various areas in Gboko town should be routinely examined to ascertain its microbiological quality and treated since they constitute the major sources of water in the study area.

Keywords: Stream; Resistance; Susceptibility; Antibiotic; Bacteria; Water

Abbreviations

C: Citrate; I: Indole; MR: Methyl-Red; VP: Voge'sproskauer; GS: Gram Stain; A: Abattoir Stream (A) ×2 point A and B; B: Nomnor Stream (B) ×2 point A and B; C: Rice Mill Stream (C) ×2 point A and B; D: Kontyen Stream (D) ×2 point A and B; CEP: Ciproflaxacin; OFX: Tarvid; NA: Nalidixic; PEF: Pefloxacin; CN: Gentamacin; AU: Augumentin; SXT: Streptomacin; PN: Ampicilin; S: Septrin

Introduction

Water is a very fundamental resource for all forms of life including animals and plants given that all cellular activities take place in fluid medium [1]. Water constitutes about seventy five percent 75% in newborns and 50% in mature animals of which 2-3% are found inside the cell [2]. The blood is comprised of 80% water and is essential for oxygen transportation to tissues and carbon dioxide away from the tissues [3].

Humans and animals drink water from a range of sources including rain water, stream water, wells etc. some of which can be contaminated by *Escherichia coli*, *Salmonella* spp. among other pathogens and chemical toxins [3-6]. Stream water that is seen to be clear and colorless to the eye is acceptable for drinking provided it is secure and free from chemicals, toxins and pathogenic microorganisms [3,4].

Furthermore, according to researchers each year 3.4 million people a good number of whom are children die from diseases that are water associated. According to United Nations Children's Fund, assessment, 4000 children die daily as an end result of contaminated water [7]. World Health Organization reports that more than 2.6 billion people have no access to clean water, which is accountable for about 2.2 billion deaths yearly, out of which 1.4 million are children which improved water quality can reduce the global disease burden by about 4% [7].

Escherichia coli and *Salmonella* spp. have been responsible for a number of food and water borne outbreak, diseases and deaths in some cases [8]. In Nigeria, residents of most rural and urban communities use untreated stream water for drinking and other household activities. Generally, the quality of stream water, in these communities is generally poor due to chemical and microbial contamination hence considered unsafe [9].

Contamination of stream water by *E. coli* and *Salmonella* spp. is not only a rising dilemma of developing countries but also for the developed countries. Stream water pollution is a major health problem worldwide resulting to nearly 1.7 billion cases of diarrheal diseases every year [10]. Almost 90% of child deaths from diarrheal diseases are unswervingly associated to contaminated water and India alone accounts for 24% of all deaths under 5 years old, among the pathogens disseminated in

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water sources, enteric pathogens such as entero-toxigenic *Escherichia coli, Shigella* spp., *Salmonella* spp., and so forth are the ones most often encountered that are accountable for array of diseases like diarrhea, dysentery, and enteric fever.

The sources of harm and health risks caused by water-borne pathogens are widely reported. In the U.S pathogens contamination is the foremost cause of water contamination. The EPA's National Water Quality Inventory Report suggests that about 53% of the assessed rivers are impaired, and a preponderance of them is contaminated by pathogens [7]. More than 4 million people die of illnesses due to infections from microorganisms, and most the cases are caused by water contamination by microorganisms. There are many forms of water use in daily life, but the greatest threat to human life occurs when there is direct contact between water and human beings, for example bathing spots where sewage is mixed into the water, office buildings that treat and recycle waste water from toilets for reuse, and water works that use river water as the water supply source [11].

Escherichia coli is a Gram-negative, facultative anaerobic, rod-shape coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherm) [12]. Most *Escherichia coli* strains are harmless, but some serotype can cause serious food poisoning in their hosts and are occasionally responsible for product recalls due to food contamination [13]. There are several pathogenic strains of *E. coli*, which are classified under entrerovirulent *Escherichia coli.* They are entero-hemorrhagic, entero-invasive, entero-toxigenic, entero-pathogenic, and entero-aggregative. *Escherichia coli* 0157:H7, the basics. It's a bacteria that causes diarrheal illness, and it's classified as an entero-hemorrhagic *Escherichia coli*. In its most severe form, it can cause hemorrhagic colitis. The reservoirs for this bacterium are cattle, deer, goats, and sheep. Humans can also be a reservoir. It is typically associated with contaminated food and water [14].

Salmonella spp. are a Gram-negative bacteria that belong to the family Enterobacteriaceae. They have a wide host range and are associated with great losses in animal production as well as with public health concerns due to their role as zoonotic and food-borne pathogens [14]. Salmonella species are bacteria that can cause diarrheal illness known as Salmonellosis. Human and animals are the reservoir, and it's typically associated with contaminated food and water. *Salmonella typhi*, it's a bacterium that causes diarrheal illness, also known as typhoid fever. And humans are the reservoir for this pathogen, *Salmonella typhi*, and prevention strategies for this pathogen include source protection, halogenations of water, and boiling of water before drinking [13]. Several methods have been developed to detect the presence of potentially low levels of *Salmonella* in food, water and clinical samples. These methods include culture based technique, DNA-based methods, immunoassays and bioassays. Standard culture methods for *Salmonella* detection involve multiple-steps which are labor intensive and time-consuming, taking 4-5 days for detection and confirmation. Furthermore, it has been reported that some of the routinely used selective enrichment broths are inhibitory toward *Salmonella* [15].

A major issue in anti-bacterial therapy is the development of resistance by sensitive strains of bacteria. Exposure of bacteria to stumpy doses of an antibiotic naturally selects for the survival of resistant organisms [16]. The introduction of antibiotics into the aquatic environments by medical therapy, agriculture and animal husbandry has led to selective pressure on bacterial population. A lot of antibiotics that are consumed end as waste, and while in wastewater, antibiotics may exert selective pressure in order to attain resistance in microorganisms. Antibiotic resistance is usually detected in wastewater, repeatedly at eminent rates and concentrations compared to surface water. Multidrug resistant bacteria in diverse environments are at the present a foremost public health misery owing to the transport of the antimicrobial-drug resistance (R) determinants in bacterial population. This is commonly mediated by genetic elements such as integrons, transposons and plasmids [16].

The occurrence of antibiotic resistance bacteria in aquatic environments has improved evidently as consequences of the widespread use of antibiotic by human. Antimicrobial substances have been employed for both treatment and prevention of bacterial diseases for several years. Because of wrong use, bacteria have developed resistance to these substances; recently, the prevalence of antimicrobial resistance has been increasing in major bacterial pathogens such as *Salmonella* [15]. Common commensal intestinal bacteria such as *Escherichia coli* and *enterococci (Enterococcus* spp.*)* are used as indicators of faecal contamination of the environment and often also as model organisms for detecting the occurrence of antibiotic resistance in both human and domestic animal populations. Recent studies showed that antimicrobial resistance occurs also in *E. coli* isolated from waters that are not directly affected by water treatment [15]. *Escherichia coli* and *Salmonella* spp. isolated from water, antibiotic resistance is a global problem and new forms of antibiotic resistance can cross international boundaries with ease. The use of antibiotics in agriculture and human treatments will maintain to increase especially in region people have less knowledge in it negative effects. The use of antibiotic is a main driver of selection pressure that contributes to resistance [1]. The cause of antibiotic resistance are complex and include human behavior at many levels of society, the consequences affect everybody in the world [1,17].

In Benue state especially in Gboko town the study area, people make use of stream waters for various activities such as cooking, washing of clothes/meats in abattoir and other domestic uses but the microbiological status of the stream water is yet to be known let alone the antibiotic susceptibility profile of *E. coli* and *Salmonella* spp. isolated from stream waters. The absence of information on the subject matter coupled with the possible risks associated with use of contaminated stream water in this part of the world is the reason for this research.

The aim of the study is to determine the antibiotic susceptibility profile of *E. coli* and *Salmonella* spp. isolated form stream water in Gboko town. The objectives of the study include:

To isolate and identify *E. coli* and *Salmonella* spp. from different stream water sources in Gboko town, using conventional culture methods.

To determine the antibiotic susceptibility of *E. coli* and *Salmonella* spp. isolated from stream water around Gboko town, using Kirby-Bauer disc diffusion method.

Routine bacteriological evaluation of water quality is required as a result of the health implication associated with consumption of contaminated stream water. The findings from this study have provided scientific data on the hygienic quality of water from streams and the antibiotic resistance pattern of the bacteria studied.

Methods

Study area

The study was conducted in Gboko metropolis Benue State. Gboko local government area is among the largest and the most populous local government areas in Benue state. It is located at latitude 8^o 30E and latitude 7^o 45N. Gboko local government area is bordered by five other local governments namely; Ushongo, Gwer, Tarka, Konshisha and Buruku local government. The majority of the local government residents have low level of formal education with few civil servant as well as trader. Gboko has a population of nearly 500,000 inhabitants, and most of them drink from stream waters. The quality of sanitation within and around the environs is very poor which could contribute to the stream water contamination.

Sterilization of equipment

Glass wares were washed with detergents using tap water and sterilized autoclaving at 121°C for 15 minutes. Sample containers and swab sticks were bought sterile and used once for each specimen. Hand gloves were bought sterile and disposed after use.

Sample collection

Ten (10) ml of water samples were collected in sterile sample containers. A total of Twenty two (22) samples were collected for an interval of three weeks, from four different locations (i.e. eight samples per week, three each from four different points of collection) and were taken to the laboratory for analysis.

Analysis of water samples

The water samples were serially diluted in tenfold to obtain a dilution of 10^{-6} and 1 ml of it was plated on a prepared Eosin methylene blue agar using the spread plate method. The inoculated plates were incubated at 37°C for 24 hours, after which suspected *Escherichia coli* and *Salmonella* spp. colonies were sub-cultured repeatedly to obtain pure cultures.

Biochemical tests

The biochemical tests; indole, citrate, methyl-red test, Voges-Proskauer test were carried out for further characterization of the isolates as described by Cheesbrough [18]. Gram staining was also carried out for proper identification of the colony.

Antibiotic susceptibility tests

Kirby-Bauer disc diffusion method adopted [3] was used to determine the antibiotic resistance pattern of *Escherichia coli* and *Salmonella* spp. against common antibiotics. One hundred micro-liter of the culture was swabbed on nutrient agar using a sterile cotton swab. Three antimicrobial disks were then placed on the surface of the agar plate at a distance to avoid overlapping of inhibition zones. The plates were incubated at 37 °C for 16-18 h and the results were interpreted as sensitive, intermediate, or resistant according to Clinical and Laboratory Standards Institute guidelines [3]. The Multiple Antibiotic Resistance (MAR) index was calculated and interpreted according to a study [3]. Using the formula: a/b, where 'a' represents the number of antibiotics to which a particular isolate was resistant and 'b' the total number of antibiotics tested.

Results

Antibiotic susceptibility profile of *Escherichia coli* and *Salmonella* spp. isolates from selected streams in Gboko town was undertaken. A total of 22 samples were collected for routine monitoring and analyzed for a period of three weeks. The response of each of the bacteria isolates to biochemical tests conducted for identification of the bacteria is presented in Tables 1-3.

Table 4 shows the result of the antibiotic susceptibility profile of *Escherichia coli* and *Salmonella* spp. Antibiotic susceptibility test on *Escherichia coli* isolated stream waters in Gboko was revealed that the highest resistance was observed in the antibiotic Ampicillin 2.6 (82.7%), followed by Gentamycin, 3.7 (76%), Ciprofloxacin (CEP), Nalidixic acid (NA), Augmentin (AU), and Streptomycin (S), had 4 (75%) respectively, while Tarvid (OFX), Pefloxacin (PEF), Seprin (SXT), had 6.6 (63.5%) and the highest susceptibility value is Ceporex (CPX) 7 (57.2%). Further antibiotic susceptibility profile of *Salmonella* spp. isolated from selected stream water in Gboko town, Tarvid (OFX), had the highest of 3 (77.9%), Augmentin (AU) 3.3 (75.5%), Gentamycin (CN) 4 (69.3%), Septrin (SXT) 5.6 (57.9%), Pefloxacin (PEF) and Streptomycin (S) had 6.6 (40.2%), while the lowest is Nalidixic acid (NA) 7 (37.1%). Most of the isolates were multi-drug resistant (Tables 1-4 and Figure 1).

Discussion

Water-borne pathogen contamination in water resources and related diseases are a major water quality concern throughout the world. The result of the present study showed that the streams in Gboko are contaminated hence unfit for human use. The study provides evidence of the presence of other pathogens in the streams with the isolation

Sample	CFU	Citrate	Indole	MR	VP	GS	Bacteria isolate
A1	52	−	+	+	+	−	*Escherichia coli*
	75	+	−	−	−	−	*Salmonella* spp.
A2	17	−	+	+	+	−	*Escherichia coli*
	19	+	−	−	−	−	*Salmonella* spp.
B1	10	+	+	+	+	−	*Escherichia coli*
	5	+	−	−	−	−	*Salmonella* spp.
B2	2.5	−	+	+	+	−	*Escherichia coli*
	72	+	−	−	−	−	*Salmonella* spp.
C1	5	+	−	−	−	−	*Escherichia coli*
C2	3	−	+	+	+	−	*Escherichia coli*
	78	+	−	−	−	−	*Salmonella* spp.
D1	54	−	+	+	+	−	*Escherichia coli*
D2	62	−	+	+	+	−	*Escherichia coli*
	25	+	−	−	−	−	*Salmonella* spp.
KEY: C: Citrate; I: Indole; MR: Methyl-red; VP: Voge'sproskauer; GS: Gram stain; A: Abattoir stream (A) ×2 point A and B; B: Nomnor stream (B) ×2 point A and B; C: Rice mill stream (C) ×2 point A and B; D: Kontyen stream (D) ×2 point A and B.							

Table 1: *Escherichia coli* and *Salmonella* spp. isolated from stream water samples in week 1 from selected streams in Gboko town.

Sample	CFU	Citrate	Indole	MR	VP	GS	Bacteria isolate
A1	45	+	−	−	−	−	*Salmonella* spp.
A2	34	+	−	−	−	−	*Salmonella* spp.
B2	44	+	−	−	−	−	*Salmonella* spp.
C1	38	−	+	+	+	−	*Escherichia coli*
	56	+	−	−	−	−	*Salmonella* spp.
C2	46	−	+	+	+	−	*Escherichia coli*
	51	+	−	−	−	−	*Salmonella* spp.
D1	2	−	+	+	+	−	*Escherichia coli*
	21	+	−	−	−	−	*Salmonella* spp.
D2	17	−	+	+	+	−	*Escherichia coli*
	23	+	−	−	−	−	*Salmonella* spp.

KEY: C: Citrate; I: Indole; MR: Methyl-red; VP: Voge'sproskauer; GS: Gram stain; A: Abattoir stream (A) ×2 point A and B; B: Nomnor stream (B) ×2 point A and B; C: Rice mill stream (C) ×2 point A and B; D: Kontyen stream (D) ×2 point A and B.

Table 2: *Escherichia coli* and *Salmonella* spp isolated from stream water samples in week 2 from selected streams in Gboko town Benue State, August 2017.

S/No	Antibiotic	*Escherichia coli* (n=16)	*Salmonella* spp (n=13)
1	CEP	3.7 (24%)	3.6 (27.6%)
2	OFX	6.6 (42%)	3 (23.1%)
3	NA	4 (25%)	7 (53.9%)
4	PEF	6 (37.5%)	6 (46.2%)
5	CN	3 (18.8%)	4 (30.7%)
6	AU	4 (25%)	3.3 (25.5%)
7	CPX	7 (43.8%)	6 (46.2%)
8	SXT	6 (37.5%)	5.6 (43.1%)
9	S	4 (25%)	6.6 (50.8%)
10	PN	2.6 (8.3%)	4 (30.7%)

KEY: CEP: Ciproflaxacin; OFX: Tarvid; NA: Nalidixic; PEF: Pefloxacin; CN: Gentamacin; AU: Augumentin; SXT: Streptomacin; PN: Ampicilin; S: Septrin.

Table 3*:* *Escherichia coli* and *Salmonella* spp. isolated from stream water samples in week 3 from selected streams in Gboko town Benue State, August 2017.

S/No	Antibiotic	*Escherichia coli* (n=16)	*Salmonella* spp (n=13)
1	CEP	3.7 (24%)	3.6 (27.6%)
2	OFX	6.6 (42%)	3 (23.1%)
3	NA	4 (25%)	7 (53.9%)
4	PEF	6 (37.5%)	6 (46.2%)
5	CN	3 (18.8%)	4 (30.7%)
6	AU	4 (25%)	3.3 (25.5%)
7	CPX	7 (43.8%)	6 (46.2%)
8	SXT	6 (37.5%)	5.6 (43.1%)
9	S	4 (25%)	6.6 (50.8%)
10	PN	2.6 (8.3%)	4 (30.7%)

KEY: CEP: Ciproflaxacin; OFX: Tarvid; NA: Nalidixic; PEF: Pefloxacin; CN: Gentamacin; AU: Augumentin; SXT: Streptomacin; PN: Ampicilin; S: Septrin.

Table 4: Antibiotic susceptibility profile of *Escherichia coli* and *Salmonella* spp. isolated from selected stream waters in Gboko town, August 2017.

of *E. coli* as an indicator organism throughout the period monitored. The presence of the isolates can be attributed to high rate of human activities within and around the streams such as swimming, washing, defecation, agricultural activities and use of the streams for waste discharge or disposal. The presence of active cases or carriers of the bacteria isolates in the community and some which are freely living in the environment can also account for their presence. Continuous use of the streams as sources of water poses risk of continuous infection without treatment and other sanitary practices.

The result obtained from the present study corroborates the research findings of other researchers reported previously in Nigeria and other parts of the world. For example, a study [18] reported on bacterial contamination of surface water sources including streams beyond WHO recommendation of '0' CFU count in any domestic water supply source. The research report isolated *E. coli* and *Salmonella* spp. among other microbes from streams. A study [19] isolated *E. coli* and *Salmonella* spp. from three streams in South Eastern Nigeria among other isolates. A study [20] isolated *E. coli* and *Salmonella* spp. whereas other researchers have isolated *E. coli* from surface water sources including streams [21,22]. Results obtained from a rural community in Cameroun showed that almost all the water samples analyzed were contaminated with different bacteria including coliforms [23,24].

Antibiotic susceptibility profile of *Escherichia coli* and *Salmonella* spp. isolated from selected streams showed high level of resistance to commonly used antibiotics. This is an indication that stream waters in Gboko town harbor resistant strains of bacteria and can be a major vehicle for its transmission to humans since water from this stream is used for domestic and recreational activities by adults and children

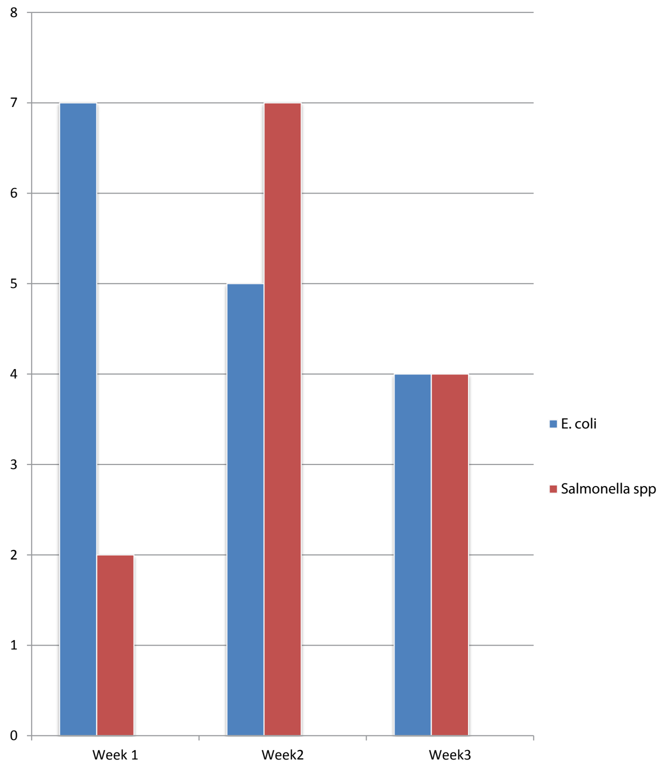

Figure 1: Bar chart showing the frequency of occurrence of *Escherichia coli* and *Salmonella* spp. isolated from stream waters in Gboko town, Benue State, August, 2017.

without prior treatment. Though most recognized sporadic cases of water borne infections may not be considered as water related but buried within the endemic level of disease circulating in the population through other routes of transmission. However recent studies [25] isolated resistant strains of *E. coli* as the highest resistant bacteria followed by *Salmonella* spp. from wounds of patients attending some hospitals in the same study area implying that the resistant strains are circulating within the population, hence the need to ensure strict adherence to hygiene and sanitation practices.

A study [26] isolated multidrug resistant bacteria from streams and well water in Ile-Ife. Antibiotic resistant *E. coli* were also isolated previously from surface waters such as streams [27,28]. Onuoha [20] reported resistance of *E. coli to* Penicillin G, Oxytetracycline, Tetracycline, Norfloxamine, Cephalothin, Sulphamethoxazole, Erythromycin, Cefuroxime sodium and *Salmonella* spp. isolated from streams resisted Streptomycin, Cefuroxime sodium, Tetracycline, Cephalothin, Cefotaxime, Nalidixic acid, Penicillin G, Sulphonamide and Cefpirome. The report however indicated that Isolates including the ones in the present study were susceptible to azithromycin, Imipenen and Norfloxamine.

Antibiotic resistance by bacteria poses a threat to virtually everyone and has been a challenge to the health sector in treatment of ailments arising from infectious bacteria and to the pharmaceutical industry for broad research geared towards discovery of more potent and alternative drugs for effective treatment.

Conclusion and Recommendations

The present result shows that adult, children, the general public and immune-compromised patients who rely on the streams studied are vulnerable to bacterial infections that have limited treatment options using basic and effective chemotherapeutic agents. Stream waters in Gboko town are contaminated with multi-drug resistant *Escherichia coli* and *Salmonella* spp. which presents a major threat to public health since the waters are commonly used for domestic activities without treatment. The use of stream waters in this area without treatment exposes the inhabitants to pathogenic bacteria which can cause severe diarrhea and other enteric infections. This may be the reason for most of the diarrhea infections experienced by most of the inhabitants of the town, since water from streams are used for irrigation of vegetable farms around the town, swimming and other domestic purposes.

Government at all levels can enact regulations against open defecation, indiscriminate dumping of wastes some of which may have been generated from health institutions in stream waters in the study area and other acts that may constitute abuse. In some parts of the town people openly defecate in streams. All these factors contribute in no small way to the contamination of water bodies.

Stream water sources from the various areas in Gboko town should be routinely examined to ascertain its microbiological quality since they constitute the major sources of water in the study area. Rising cases of antibiotic resistance is a major concern worldwide and is evident in this study area therefore; public awareness and dissemination of this research finding to health institutions and Government authorities for appropriate decisions will be of great benefit to the residents.

Water treatment campaigns should be held in the area to enlighten the residents on safe water treatment measures and the menace of drug resistance by bacteria from the streams and the strategies to improve health.

Availability of Data and Materials

All the generated and analyzed data during the study are included in the article.

Author's Contributions

This work was carried out in collaboration between all authors. Author Ichor, Tersagh designed the project in collaboration with the co-authors and wrote out the article to this present standard. He planned and led the research to execution, analyzed data and effected all the corrections as required by the reviewers. Author Terwase, Aondona Jerry and Aburu Francis Esidene assisted in the design, execution and also contributed in the literature search, drafting of the article and statistical analysis. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict or competing interest either financial or non-financial.

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