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Multidrug-Resistant Enteric Bacteria in the Water Sources of Kalgo Metropolis, Nigeria

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ABSTRACT

People in rural and impoverished regions are more likely to contract water-related illnesses such as cholera, typhoid fever, bacillary dysentery, and diarrhea because they drink polluted water at home. The main drinking water sources in Kalgo City, Nigeria, were tested for the presence of enteric bacteria. Three water samples were taken from three different locations inside the municipality using sterile procedures in triplicate. After a series of dilutions, the specimens were placed on nutrient agar, and the total number of viable bacteria was determined. Isolation and identification of the intestinal bacteria were carried out using conventional methods. Using the Kirby Bauer disk diffusion technique, the isolates were tested for antibiotic susceptibility. We used millimeters (mm) to measure the inhibitory zones. The water samples yielded the isolation of seven(7) distinct bacterial species. Shigella, Serratia, Klebsiella, Escherichia coli, Proteus, Yersinia, and Salmonella were among the pathogens that were isolated. The two most common types of these bacterial isolates were E. coli (29.63%) and Salmonella spp. (24.93%). Serratia spp. had the lowest frequency at 3.9%. Bacteria including E. coli, Salmonella, Klebsiella, Shigella, and Serratia were shown to be resistant to ampicillin, according to the sensitivity profile. On the other hand, Yersinia spp. demonstrated a moderate degree of susceptibility, whereas Proteus spp. showed a high level. Evidence of multidrug-resistant enteric bacteria in water samples emphasizes the need to evaluate and purify water before using it to enhance its quality.

Keywords: enteric bacteria, multidrug-resistant, Kalgo metropolis, Nigeria, water contamination, water sources

1. INTRODUCTION

Water is considered a universal solvent.Its safety, adequacy, and accessibility must be assured at the home level. Access to quality drinking water can provide substantial health advantages. Continued efforts should be undertaken to guarantee that clean drinking water is accessible to everyone [1]. The most common health concern associated with drinking water is pollution from human or animal excreta carrying dangerous germs. Domestic usage of polluted water can cause many illnesses [2]. 4 billion (estimated)

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cases of diarrhoeaare caused by contaminated drinking water annually, representing 5.7% of the GDB (global disease burden) [3]. Several waterborne, water-based, and sanitary-associated diseases are linked to the quantity and quality of water and sanitation that consumers can access, according to a World Bank review of 28 studies [4]. Most Nigerians living in remote locations lack access to clean and dependable sanitary services. Furthermore, most homes lack an appropriate grasp of sanitary practices including food, water, and personal hygiene [5].

In addition to difficulty in acquiring safe and sanitary drinking water, metropolitan water can be polluted with pathogens at its sources, during distribution, transit, and owing to improper handling in residences or at the workplace [6]. Human and animal excrement are the most prevalent causes of microbial contamination in water. The most serious microbiological dangers come from consuming water polluted with human or animal excrement.Such water is contaminated with dangerous bacteria, viruses, protozoa, and helminths [1, 7].

A short-term rise in pathogen content in drinking water may significantly raise illness risks and cause waterborne disease outbreaks [8]. Many bacteria that cause serious infections, such as typhoid fever, cholera, and dysentery, are directly linked to polluted drinking water. A low-cost and environmentally friendly technology called as a bacterium indicator can be used to indirectly determine the contamination level of drinking water. Coliform bacteria, found in the environment and the human and animal intestines, are one kind of bacterium that can be detected. The presence of these bacteria in water over a particular level indicates the hazards [9]. Water consumed by the humans should not include E. coli more than 100mL in water sample [10]. The presence of 10 E. coli counts per 100 ml of the water sample is okay, but regular sanitary inspections are required if it is not chlorinated [6].

Salmonella, E. coli, Yersinia pestis, Klebsiella, and Shigella are all members of the large Gram-negative bacterial family known as enteric bacteria. This family also contains harmless symbionts. This family also includes Proteus, Enterobacter, Serratia, and Citrobacter, all of which cause sickness. Enteric bacteria are often found in the intestines of animals and humans [11]. Some are pathogenic, causing disease in particular animal species, whereas others may exist in the gut without producing any health issues in a healthy person. The enteric bacteria family that causes water pollution is made up of individuals from the order Enterobacteriales of the class Gamma proteobacteria in the phylum Proteobacteria [12].

The length of an Enterobacteriaceae rod might vary between 1 and 5 µm. On blood agar, they often show up as gray colonies of medium to large size; however, certain species, including Serratia marcescens, are capable of expressing colors. As a means of cell adhesion, the majority of Enterobacteriaceae possess peritrichous type I fimbriae. Enterotoxins are produced by specific types of bacteria.As the cell dies, these substances released into the surrounding fluid cause the cell wall to disintegrate. When cells of some Enterobacteriaceae break down, their toxic byproducts enter the bloodstream and trigger a cascade of events that include inflammation and vasodilation [13]. As a result, bacteria's creation of endotoxins may be one of the major causes and methods leading to their multi-drug resistance. Some species' strains, such as E.

coli, are innocuous commensals.Others are significant human and animal diseases, while certain types are harmful to plants and insects. Their widespread distribution shows that certain organisms of the Enterobacteriaceae infiltrate the food chain [14].

Dumpsites are the most affordable and extensively utilized technique of trash disposal [15]. However, studies have connected dumpsites to groundwater pollution, prompting the safety evaluation of groundwater supplies near waste sites [16]. The existence of antibiotic residues in the environment is concerning because drugs may lead to the emergence of resistant microorganisms and impose selection pressure [17]. There is strong evidence that wastewater from hospitals, cities, farms, and aquariums is a major contributor to antibiotic resistance and other antibiotic-related problems in aquatic environments [18]. Waterborne diseases are on the rise due to the high prevalence of multidrug-resistant enteric bacteria and other contaminants in our water supply. Regular water quality assessments are essential for reducing this risk, promoting better public hygiene, and enhancing the standard of living in both urban and rural areas, especially in developing nations.

2. MATERIALS AND METHODS

2.1. Study Area

This study was conducted in Kalgo Metropolis,Kebbi State, Northwestern Nigeria. Kalgo is situated 10 km away from BirninKebbi, the capital of Kebbi State, located at latitude 12° 27' 57.8808" N and longitude 4° 11' 58.2864" E [19]. It is one of the emerging metropolises around Birnin Kebbi as a result of the development of Federal University Birnin Kebbi, where the number of students, faculty, and businesspeople grows on a regular basis. This population growth, as well as other human activities, represent a significant risk to land and water resources.

2.2. Sample Collection and Handling

From October to November 2022, 27 samples (three in duplicate, each from a separate place in Dutsin Dodo, TudunIllela, and Shiyar Dan Fili) were gathered around the Kalgo city. Water samples (100 ml) were collected aseptically from a well, river, and borehole using a sterile funnel and transferred to a clean container. After collecting and transferring samples into a sterile container, it was firmly sealed and delivered to the laboratory. All samples obtained were labeled with the sample number, date of collection, and source. The sealed samples were subsequently delivered to the lab for examination.

2.3. Identification of Bacterial Isolates

Using the pour plate method, 100 µl of water samples that had been serially diluted were infected onto nutrient agar plates. The plates were then incubated at 37°C for 24 hours. The colonies for each sample were counted and expressed in colony-forming units per milliliter (cfu/ml) according to the procedures given by Palanisamy et al. [17], Subculturing the colonies allowed for the extraction of pure colonies. Isolates of bacteria were identified by employing conventional biochemical techniques [20].

2.4. Antimicrobial Susceptibility Testing

To determine susceptibility, we followed the guidelines set out by the National Committee for Clinical and Laboratory Standards and used the Kirby Bauer disk diffusion technique. After transferring bacterial colonies from nutritional agar to sterile normal saline, the resulting volume was four to five milliliters (ml). A turbidity level similar to the 0.5 MacFarland criterion was achieved by visually adjusting

the suspension. The inoculum was applied to the whole surface of the Muller Hinton agar plate with a sterile swab stick. To achieve uniform dispersal, the plate was rotated around 60 degrees between streakings. The contaminated plates were allowed to stand for at least 3 minutes, but no more than 15 minutes, before the disks were applied.The disk was gently squeezed using sterile forceps to make contact with the medium. Commercial antibiotic disks (Abtex Biological Ltd, Liverpool, United Kingdom) were utilized. These included tarivid (10µg), reflacine (10µg), ciproflox (10µg), gentamycin (10µg), streptomycin (30µg), ceporex (10µg), augumentin (30µg), aalidixic acid (30µg), septrin (30µg), and amplicin (30µg).

3. RESULTS

3.1. Total Bacterial Load of the Isolates

The results for bacterial load from water samples ranged from $3.1x10^{-5}$ to $2.6x10^{-6}$ in Dutsin dodo, $2.0x10^{-4}$ to $2.2x10^{-5}$ in TudunIllela, and $1.0x10^{-6}$ to $3.0x10^{-6}$ in Shiyar Dan Fili,where Shiyar Dan fili (river water) was found to have the highest bacterial load. The high bacterial levels in water sources can be attributed to several factors, including uncovered water sources and exposure to dust, as well as the use of contaminated toilet articles to fetch water and the lack of proper sanitation of reservoirs. This is demonstrated in Table 1.

3.2. Identification of Bacterial Isolate

In this study, 27 (3 samples in each area in the triplicate) bacteria were isolated from 3 locations inthe Kalgo metropolis. The isolates identified were *Shigella* spp.,*Serratia* spp., *Klebsiella* spp., *Escherichia coli*, *Proteus* spp., *Yersinia* spp, and *Salmonella* spp. Out of the 27 bacteria genera isolated, *E.coli and Salmonella* spp.were found to have the highest occurence, while *Serratia* spp. occurred the least, as shown in (Table 2).

Table1. Total Viable Plate Count of BacterialIsolates (cfu/ml)

Samples/ Location/ Sources	No of Colonies	Total Plate Count (cfu/ml)
Dutsin Dodo/Borehole		
1	180	$1.8x10^{-4}$
2	29	$2.9x10^{-4}$
3	232	$2.3x10^{-4}$
4	221	$2.2x10^{-4}$
5	262	$2.6x10^{-6}$
6	224	$2.2x10^{-6}$
7	88	$8.8x10^{-3}$
8	71	$7.1x10^{-2}$
9	31	$3.1x10^{-5}$
TudunIllela/Well		
10	50	$5.0x10^{-4}$
11	20	$2.0x10^{-4}$
12	56	$5.6x10^{-4}$
13	160	$1.6x10^{-5}$
14	170	$1.7x10^{-5}$
15	200	$2.0x10^{-5}$
16	210	$2.1x10^{-5}$
17	225	$2.2x10^{-5}$
18	67	$6.7x10^{-4}$
Shiyar Dan Fili/Rivers		
19	200	$2.0x10^{-6}$
20	100	$1.0x10^{-6}$
21	250	$2.5x10^{-6}$
22	100	$1.0x10^{-6}$
23	285	$2.8x10^{-4}$
24	303	$3.0x10^{-6}$
25	190	$1.9x10^{-4}$
26	155	$1.5x10^{-4}$
27	305	$3.0x10^{-6}$

Table2. Identification of Bacterial Isolates from Water Samples

S/N	Gram	Glucose	Sucrose	Lactose	Motility	Indole	urease	Citrate	H_2S	Gas	Isolate
1	-ve Rod	+	-	-	+	-	-	-	+	-	*Salmonella* spp.
2	-ve Rod	+	+	+	-	-	+	+	-	+	*Klebseilla* spp.
3	-ve Rod	+	+	+	+	+	-	-	-	+	*E.coli*
4	-ve Rod	-	+	-	-	-	-	-	-	-	*Shigella* spp.
5	-ve Rod	+	-	-	-	-	-	-	-	-	*Yersinia* spp.
6	-ve Rod	+	+	-	+	-	+	+	-	-	*Serratia* spp.
7	-ve Rod	+	-	-	+	-	+	+	+	+	*Proteus* spp.

3.3. Frequency of Occurrence of Enteric Bacteria Isolated from Water per Area

The study revealed that in area 1 (Dutsin Dodo), the water sample analyzed showed a high frequency of occurrence of bacteria, with *E. coli* having the highest percentage at 33.33% (3 occurrences). In contrast,*Klebsiella*, *Yersinia*, and*Serratia*were found to have the lowest frequency of 1(11.11%) each. In area 2 (TudunIllela),*E. coli*had the highest frequency of occurrence of 3 (33.33%), while*Shigella* and *Yersinia*were found to have the lowest frequency of 1(11.11%) each. While, area 3 (Shiyar Dan Fili) showed the highest frequency of *Salmonella* with 3 (33.33%) and the lowest frequency of occurrence of *Klebsiella* and *Yersinia* with 1(11.11%) each, as shown in Table 3.

The total frequency of occurrence of isolates indicates that out of the seven (7) bacteria genera isolated, *E. coli* (29.63%) and *Salmonella* spp.(25.93%)showed the highest percentage of occurrence, respectively. In contrast,*Serratia* spp.showed the lowest percentageof occurrence (3.70%), as illustrated in the pie chart.

Table3. Frequency of Occurrence of Enteric Bacteria Isolated from Water Sample per Area

Area	*E.coli*	*Salmonella* spp.	*Klebseilla* spp.	*Shigella* spp.	*Yersinia* spp.	*Proteus* spp.	*Serratia* spp.
Area 1(Dutsin Dodo)	3(33.33%)	2 (22.22%)	1(11.11%)	1(11.11%)	1(11.11%)	0 (0%)	1 (11.11%)
Area 2 (TudunIllela	3(33.33%)	2 (22.22%)	0 (0%)	1(11.11%)	1(11.11%)	2(22.22%)	0 (0%)
Area 3 (Shiyar Dan Fili)	(22.22%)	3 (33.33%)	1 (11.11%	0 (0%)	1(11.11%)	2(22.22%)	0 (0%)

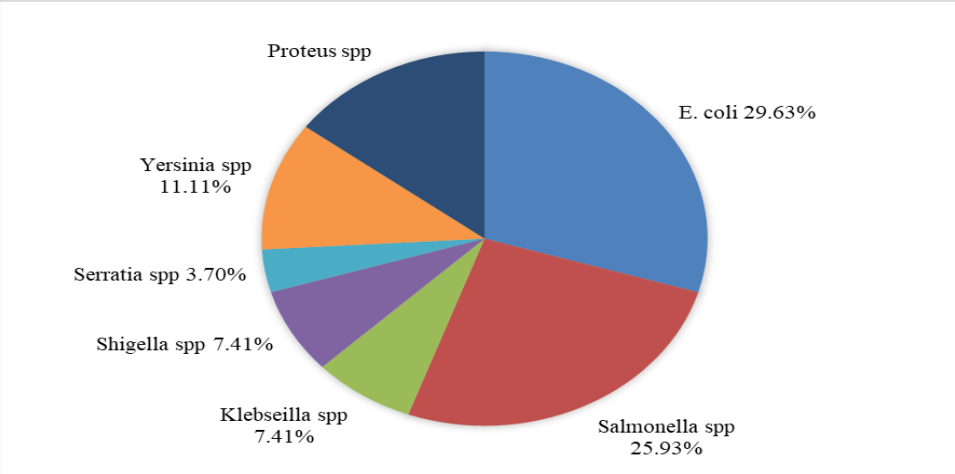

Figure 1. Percentage of Occurance of Bacterial Isolates

Table 4. Zones of Inhibition in Millimeters(mm) Obtained using the Kirby Bauer Disk Diffusion Method

Isolate	CN	AU	CPX	SXT	S	PN	CEP	OFX	NA	PEF
E.coli	S	S	I	I	R	R	R	S	I	S
Salmonella spp.	I	S	I	S	S	R	I	S	S	S
Klebsiella spp.	S	S	R	S	S	R	I	S	S	I
Shigella spp.	S	S	I	S	R	R	S	S	S	S
Serratia spp.	S	S	I	I	S	R	R	S	S	I
Proteus spp.	R	I	I	R	S	S	S	I	I	R
Yersinia spp.	I	R	S	R	I	I	S	S	R	S

Key: S: susceptible. I: intermediate, R: resistance, CN: gentamycin, SXT: septrin, CEP: ceporex , PEF: reflacin, AU: augumentin, S: streptomycin, OFX: tarivid, CPXciproflox, PN: ampicillin, NA: nalidixicacid

4. DISCUSSION

Numerous microbes have apparently been discovered in polluted water. Monitoring and identifying indicator organisms and disease-causing microbes is an important aspect of sanitary microbiology. Bacteria present in the digestive system seldom thrive in watery environments. They continue to experience physiological stress and progressively lose their capacity to establish colonies on various sensitivity mediums. Bacterial transmission often occurs via the fecal-oral channel, which can be direct or indirect, such as when people come into touch with healthcare personnel. Inadequate hygiene is a significant risk factor for contracting and being infected with enteric bacteria. The frequency of these intestinal microorganisms changes dramatically from person to person, indicating a multi-factor driver.

The bacterial loads in water samples varied, with Shiyar Dan Fili (river water) having the greatest load, ranging from 1.0x10-6 to 3.0x10-6. In comparison, Dutsin dodo varied from 3.1x10-5 to 2.6x10-6, while Tudun Illela ranged from

2.0x10-4 to 2.2x10-5. The results showed that all water samples tested from the river were significantly polluted. This conclusion is consistent with the study done in Sagamu, Nigeria, which indicated that the majority of the river water (72%) was polluted with E. coli, Salmonella spp., Proteus spp., and Klebsiella spp., respectively [21].

The presence of coliform bacteria in drinking water is used to identify fecal or other potential causes of pollution. In the current investigation, seven (07) bacterial species were recovered from the water samples. Out of the 27 bacteria genera identified, E. coli (29.63%) and salmonella spp. (25.93%) had the highest proportion of occurrence, while Serratiamercescens (3.70%) had the lowest. This conclusion is consistent with previous findings by Aliero et al. [22] on the antibiotic resistance pattern of coliform bacteria isolated from slaughterhouse effluent in the Jega local government.Jega is located 10 kilometers ahead of the Kalgo city and contains three Gramnegative bacteria: E. coli, Pseudomonas aeruginosa, and Enterobacteraerogenes.E. coli was the most often isolated pathogen in slaughterhouse wastewater 39 (45.8%).

This study further correlates with the bacteriological and physicochemical analyses of Aliero dam water by Gulumbe et al. [23]. The analyses revealed that Yersinia enterocolitica and Staphylococcus aureus had the highest percentage of occurrence (20%), Bacillus megaterium, Salmonella sp., and Escherichia coli had 12.5% occurrence, and Klebsiella, Vibrio, and Shigella spp. had the lowest percentage of occurrence (7.5% each). This association might be explained by the connection between the canals and rivers from Aleiro to Jega and the Kalgo metropolis river, since Aleiro and Jega are physically ahead of the Kalgo metropolis. The presence of these creatures can be related to human activity, as humans serve as reservoirs for a variety of species. This is also consistent with the findings of Ouseph [24]. This study investigated the occurrence of intestinal bacteria in healthy human carriers. He observed that healthy people contain a large variety of enteric bacteria, including Salmonella spp., Shigella spp., Citrobacter spp., Yersinia spp., E. coli, Klebsiella spp., Proteus, and Enterobacter spp., with E. coli and Salmonella being the most often isolated bugs

Table 4 reveals that all organisms' resistance to various antibiotics is troublesome, particularly resistance to three or more medicines, which indicates multidrug resistance. The data revealed that ampicillin, with 12.5% susceptible, 12.5% intermediate, and 75% resistant isolates, is the antibiotic with the most resistance isolates. Ofloxacin, with 85.7% susceptible and 14.38% intermediate isolates, is the antibiotic with the most susceptible isolates. These results are consistent with the findings of a research done in Ghana, where a significant rate of resistance to ampicillin and tetracycline was identified [25]. Epidemiological research shows that the rising incidence of resistance to tetracycline, ampicillin, and many other antibiotics is directly related to their use [26, 27].

It was also discovered that none of the isolates examined were sensitive to all of the antimicrobial medicines utilized in this investigation. This is reason for worry because many practitioners rely on quinolones to treat.Gram-negative microorganisms and drug resistance [28, 29]. Ciprofloxacin resistance was 25.8% in Portugal and 24.3% in Italy, but it was 15.2% in Germany and 6.8% in the Netherlands [30]. Similarly, in a study

conducted by Yusuf et al. [8] in Nigeria, up to 18% resistance to ciprofloxacin was reported. In another study, Thomas et al. [30] shown that all K. pneumonia were sensitive to ciprofloxacin. The rising resistance to various antimicrobial medications has been attributed to their incorrect use (including overuse, abuse, poor dose, and noncompliance with the treatment period), which leads to selection pressure. According to Sougakoff and Jarlier [31] the overuse and misuse of antimicrobial drugs for growth promotion and illness prevention has put a selective pressure on bacteria, resulting in increased resistance.To limit the danger of contamination of water sources and consumers, sanitary microbiologists must continue to monitor and detect signs and disease-causing bacteria [32].

4.1. Conclusion

The current study found that the bacterial load in drinking water collected from three sources in the Kalgo metropolis varied, with the highest load in Shiyar Dan Fili (river water). The most common indicator organisms for faecal contamination were *Shigella* spp., *Serratia* spp., *Klebsiella* spp., *E.coli*, *Proteus* spp., *Yersinia* spp., and *Salmonella*spp.*E. coli* and *Salmonella* spp.had the highest percentage of occurrence among the 27 isolated bacteria genera. The results also showed that none of the isolates were susceptible to all the tested antibiotics, indicating a resistance profile that remains a cause for concern. This poses a threat to human health and calls for further investigation and measures to improve drinking water quality.

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