Original Research Article

BACTERIOLOGICAL EVALUATION OF SURFACE AND GROUNDWATER USED FOR DOMESTIC PURPOSES IN IBADAN.

ABSTRACT

The right to safe and clean drinking water is a human right, unfortunately many communities rely on unsafe and contaminated water for drinking and domestic purposes. Through improper sewage and hospital waste disposal, pathogenic and antibiotic resistant bacteria have found their way to natural water sources used by humans. In this study we aim to profile pathogenic bacteria and resistant *Escherichia coli (E. coli)* from surface and ground water sources in three local government areas of Oyo state. 30 water samples were collected randomly from the selected local government areas. Bacteriological, biochemical and antibiotic-resistant analysis was carried out on the isolated bacteria from the water samples. *E. coli* (28.30%) and *Kleb pneumoniae* (23.77%) were the predominant bacteria isolates, while Shigella (16.22%) and Salmonella (16.22%) also had substantial percentages.Nineteen *E. coli* was isolated and identified, with high antibiotic resistance rates observed in nalidixic Acid (100%), augmentin (100%), ampiclox (94.74%) and third-generation cephalosporins. Conversely, moderate levels of resistance were observed in nitrofurantoin (55.55%), cefepime (57.89%), and second-generation fluoroquinolones. This study reveals substantial pathogenic bacteria and antibiotic resistant*E. coli* in water samples which endangers the health of communities' dependent on these water sources and exposes the inhabitants to antibiotic-resistant organisms fromcontaminated water used for domestic purposes.It is crucial to put in place water treatment measures and quality monitoring programmes in order to guarantee that the populace has access to clean and safe drinking water.

Keywords: Contaminated water, *Escherichia coli,* antibiotic resistance, Ibadan

1.0 INTRODUCTION

Water, one of the pillars from which life originates and without which life would cease to exist; is a fundamental resource for various human activities. Over the years, humans have devised diverse means of obtaining fresh water from rivers, lakes, streams and underground water sources. But, the demand for fresh water has skyrocketed due to the explosion in population growth and environmental pollution majorly caused by industrial activities, leading to a decline in fresh water resources[1]. The importance of clean water is buttressed by the diverse diseases caused by pathogens that are transmitted through unsafe water[2]. In its natural state water contains impurities and microbes that render it unsafe for human consumption. These impurities and

microbes are also introduced to fresh water sources through human activities thereby endangering the health of communities that depend on such sources[3]. It is pertinent that water meant for human consumption be treated to avoid disease outbreak[4]. The high prevalence of water-borne disease—cholera, dysentery and typhoid—in developing countries like Nigeria is primarily attributed to unsafe, untreated or contaminated water[5].Despite its significance, water resource management remains inadequate on a global scale. Ensuring access to safe drinking water is of paramount importance for both rural and urban populations in order to mitigate health risks[6]. It is now common knowledge that natural ecosystems are reservoirs of antibiotic resistant genes, particularly domestic water bodies that receive industrial, hospital and animal waste[7]. The usage of untreated water containing hazardous waste has a high potential of housing and transmitting pathogenic and resistant microbes to individuals and communities. This is of great public health concern, considering the significant threat antibiotic resistant bacteria poses to global healthcare[7].*Escherichia coli (E. coli)* are a highly suitable species for investigating the transmission of antimicrobial resistance through manure, animal feces, inadequately treated wastewater, and sewage overflow caused by heavy rainfall contamination of water due to their ubiquitous nature[8].

In order to mitigate the dissemination of bacterial pathogens in domestic water sources, it is imperative to evaluate the impact of water treatment plants on the environment. This will shed light on the potential sources of environmental contamination and routes of exposure.Assessing water consumed in communities for the presence of disease causing pathogens is a necessity, considering the poor understanding on antimicrobial resistant reservoirs in Nigeria.Therefore, this study aims to isolate and identify the presence of bacterial pathogens and investigate the pattern of antimicrobial resistance patterns in *E. coli* from both surface and underground water used for domestic purposes in selected locations in Ibadan, Oyo State.

2.0 METHODOLOGY

2.1 Study area and work site

This study took place in three local government areas—Ibadan South East (Mapo), Ibadan North West (Onireke) and Oluyole Local Government, Ibadan, Oyo State. This water analysis was carried out at the microbiology laboratory of the department of biological science, Lead City University, Ibadan, Oyo State.

2.2 Sample size determination

A total of 30 water samples from ground water (borehole) and surface water (stream) was collected randomly at three different selected local government areas in Ibadan, Oyo State for analysis.

2.3 Collection and storage of samples

The water sample (300 ml) was collected into a sterile bottle and each was duplicated at each site of collection. All samples were placed with ice inside the cooler and transported to the laboratory for both microbiological and physiochemical analysis.

2.4 Bacteriological analysis of the water samples

Preparation of culture media

MacConkey, Nutrient and Eosin methylene blue (EMB) agar were prepared under aseptic conditions and stored according to the manufacturer's instructions.

Isolation of *E. coli* using membrane filtration techniques according to the standard for the examination of waste water.

All parts of the membrane filtration machine were sterilized by autoclaving at 121^{o}C for 15 min. A sterile forceps was utilized to handle the filter paper, which was then placed onto the filter housing machine. The top half of the assembly was securely clamped to prevent any contamination. A volume of approximately 100 ml of water sample was introduced into the filtration system, and the vacuum was activated to facilitate the passage of water through a specialized porous membrane designed to capture microorganisms larger than 0.25 µm. After all the water had successfully passed through the membrane, the vacuum was deactivated and the top half of the filter was removed. A sterile forceps was used to carefully remove the filter, which was then placed onto the center of a solidified selective media. The plate was subsequently incubated at a temperature of 37°C for a period of 24 to 48 hours.

2.4.1 Sub culture

Platinum wire loop was used to pick the colony; from selected EMB plate and streaked onto the nutrient plate. The petri dish was then incubated at a temperature of 37ºC for a period of 24 to 48 hours, to allow the discrete growth of the isolated organism.

2.4.2 Identification and characterization of the isolate

Presumptive Escherichia coli isolates exhibiting a greenish metallic sheen on EMB agar were subjected to Gram differentiation and biochemical identification methods as outlined by Odonkor and Ampofo (2013). The biochemical tests conducted encompassed citrate utilization, indole production, motility, and triple sugar ion tests.

2.4.3 Gram staining

A tiny portion of the selected isolate was carefully picked using a sterile inoculating loop. This portion was then homogenized in a drop of distilled water on a clean sterile microscopic slide. The resulting mixture was spread in a circular motion using the inoculating loop and heat fixed. The smear was then gently flooded with a 1% solution of methylene blue for one minute and rinsed with distilled water. A few drops of Lugol's solution were then added and allowed to remain on the smear for one minute before being rinsed with distilled water. The smear was afterward decolorized using 95% ethanol. The slide was immediately rinsed and then counter stained with a 0.5% solution of safranin red for approximately 60 seconds. The smear was once

again rinsed with distilled water and air dried. Lastly, a few drops of oil immersion were added to the smear and then examined under a microscope at x100 objective.

2.5 Biochemical Tests

2.5.1 Citrate Utilization Test

The isolate was inoculated on Simmon's citrate mediumand incubated at 37°C for 24 hours. A positive reaction was indicated by a color change from the initial greenish hue of the media to a Prussian blue color.

2.5.2 Indole Production Test

The isolate was inoculated in a bijou bottle containing tryptone water and incubated at 37^{o}C for 24 hours. Few drops of Kovac's reagent was then added to the inoculated tryptone water and examined for approximately 10 minutes. A positive reaction was indicated by the appearance of a red coloration at the surface.

2.5.3 Motility Test

Sulfur Indole Motility (SIM) tubes were inoculated with a single stab at the base of the tube and were then incubated for 24 hours at 37^{o}C. The positive tube demonstrated radial growth of the inoculum from the stab mark, resulting in the entire tube becoming turbid.

2.5.4 Triple Sugar Ion Agar

TSI agar slants were prepared, and a sterile needle was used to pick a colony of the test isolate. The needle was then stabbed to the bottom of the slant while the surface of the slant was streaked. The slants were incubated overnight at 37^{o}C for 24 hours, and the tubes were examined and the observations were recorded.

2.6 Antibiotic Resistance Profile

The isolates were tested for susceptibility to 12 different antibiotics that are relevant in both veterinary and human clinical settings. The antibiotics tested included Ampiclox (10 µg), Ceftriaxone (25 µg), Imipenem (10 µg), Ofloxacin (5 µg), Nalidixic acid (30 µg), Ceftriaxone (45 µg), Cefuroxime (30 µg), Nitrofuranton (300 µg), Augmentin (30 µg), Levofloxacin (5 µg), Cefepime (30 µg), and Gentamicin (10 µg).The selection of these antibiotics was based on their importance in treating *E. coli* infections in humans and animals.

A direct broth suspension was made from a discrete colony selected from a 24-hour culture using sterile peptone water. The suspension was adjusted to match the 0.5 McFarland standards for the study.The dried surface of a Mueller-Hinton agar plate was then inoculated by pouring the standardized inoculum onto the plate and rotating it to ensure even distribution. Excess inoculum was removed by decanting it into a bowl containing disinfectant liquid. The surface of the inoculated plates was allowed to dry before applying the drug-impregnated disks. The antibiotic

disks were aseptically dispensed onto the surface of the inoculated agar plate, and each disk was pressed down to ensure complete contact with the agar surface. The plates were then inverted and incubated at 35°C for 18-24 hours, and the diameter of the zone of inhibition was measured and recorded.The results of the susceptibility testing were interpreted using the standard interpretative charts provided by the CLSI in 2012.

2.7 Statistical analysis

The data obtained were to analysis of variance (ANOVA) at $p \leq 0.01$ using statistical package for social sciences version 23.

3.0 RESULTS

A total of 71 bacteria were isolated from the 30 water samples in the three local government areas in Ibadan as depicted in Figure 1. *E. coli* had the highest number of isolates 19 (26.76%), while *Klebsiella pneumoniae (K. pneumoniae)* at 15 (21.13%) was the second most prominent bacteria isolated. *Shigella* at 12 (16.90%), *Salmonella*at 11 (15.49%), *Bacillus subtilis* at 9 (12.68%), *Staphylococcus aureus*3 (4.23%) and *Streptococcus spp* 2 (2.82%) were the other bacteria isolated after analysis.

The bacteria analysis for Local Government 1 indicated that *E. coli* was the most commonly found bacterium, with 28.57% of the isolates, as depicted in Figure 2. 25% of the samples yielded *K. pneumoniae* while 17.86% of the isolates yielded *Salmonella* and *Shigella*each. The lowest prevalence was about 3.57% in *Bacillus subtilis, Staphylococcus aureus* and *Streptococcus spp*each. Figure 3 depicts the bacteria distributionin Local Government 2, 30% of the isolates yielded *E.coli*, while 20% of the isolates yielded *K. pneumoniae* and 15% of the isolates yielded *Bacillus subtilis, Salmonella* and *Shigella*. 5% of the isolates yielded *Staphylococcus aureus* and none of the isolates yielded *Streptococcus spp*.In Local Government 3, *E.coli* and *K. pneumoniae* had the highest percentage of yielding isolates of about 26.32% each, while 15.79% of the isolates yielded *Salmonella* and *Shigella*each. 10.53% of the isolates yielded*Bacillus subtilis*, while5.26% of the isolates yielded*Staphylococcus aureus* and none of the isolates yielded *Streptococcus spp* as shown in Figure 4.

Nineteen *E. coli* wereisolated and identified (Table 1). The antibiotics susceptibility result revealed that all (100%) *E. coli* strains exhibited resistance to Nalidixic Acid (30 µg) and Augmentin (30 µg) as shown in figure 4. Furthermore, higher antibiotic resistance pattern rates were observed in the third generation of Cephalosporins e.g. Cefotaxime (25 µg) at 73.68%, Cefexime (5 µg) at 68.42%, Ceftriaxone (45 µg) at 73.68%, and Cefuroxime (30 µg) at 94.74%. In contrast, the fourth generation of Cephalosporin, Cefepime (30 µg), showed a resistance rate of 57.89%. Significant antibiotic resistance of the isolated *E. coli* was also recorded for Ampiclox (10 µg) at 94.74%, Levofloxacin (5 µg) at 63.16%, Imipenem (10 µg) at 78.95%, and

Gentamicin (10 µg) at 68.42% while moderate resistance was observed in Nitrofurantoin (300 µg) at 55.56% and Ofloxacin (5 µg) at 57.89%.

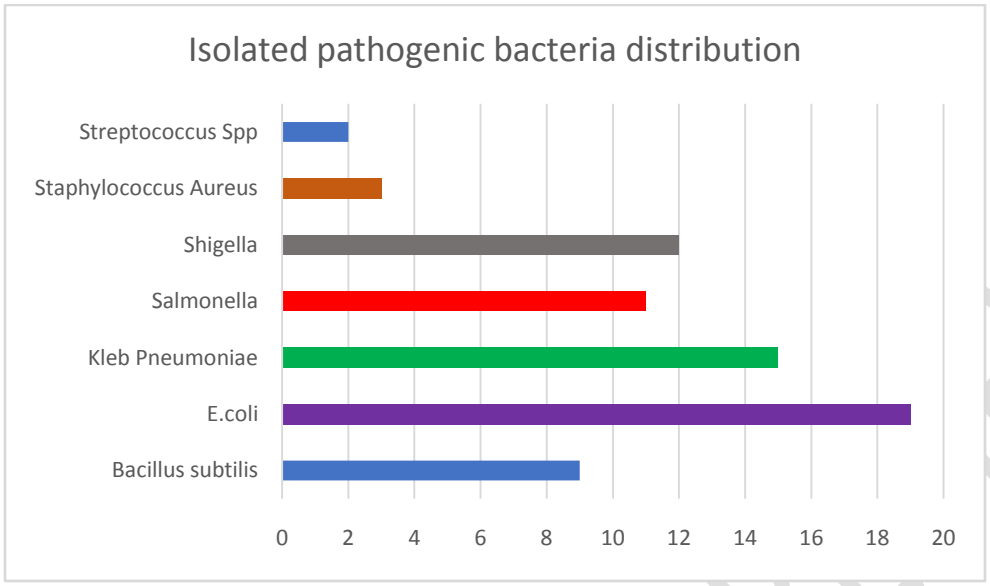

Figure 1: Distribution of isolated bacteria from the 30 water samples

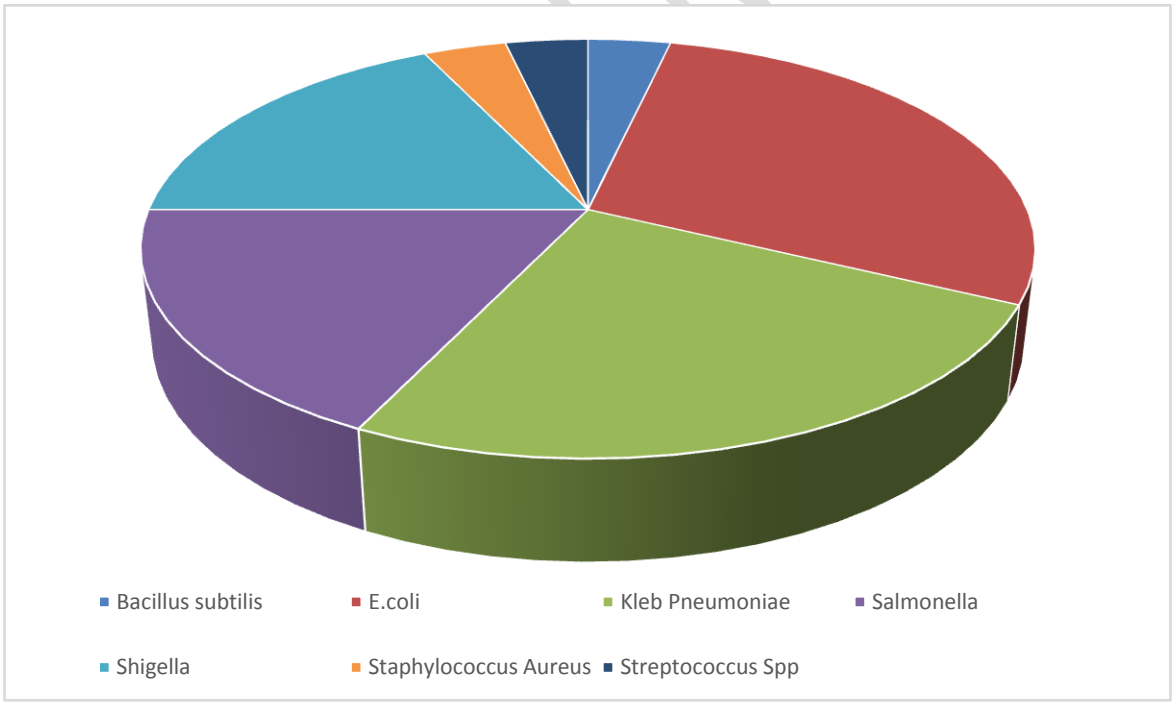

Figure 2: The isolated bacteria from the water sample at Local Government 1

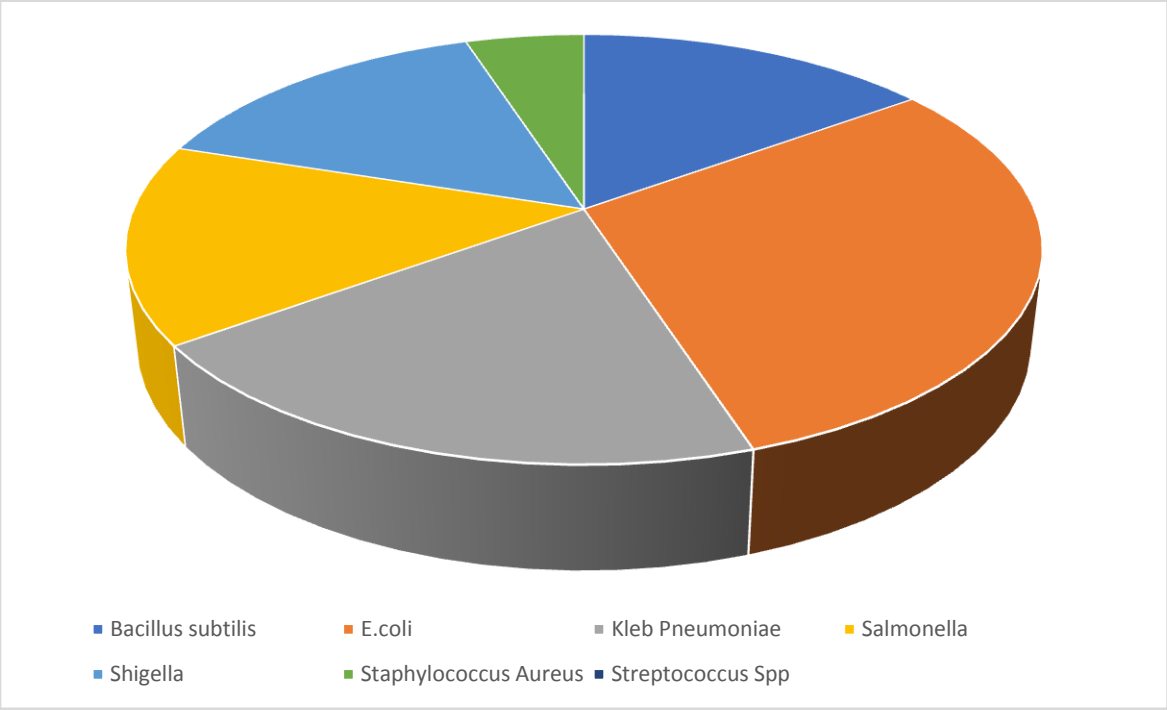

Figure 3: The isolated bacteria from the water sample at Local Government 2

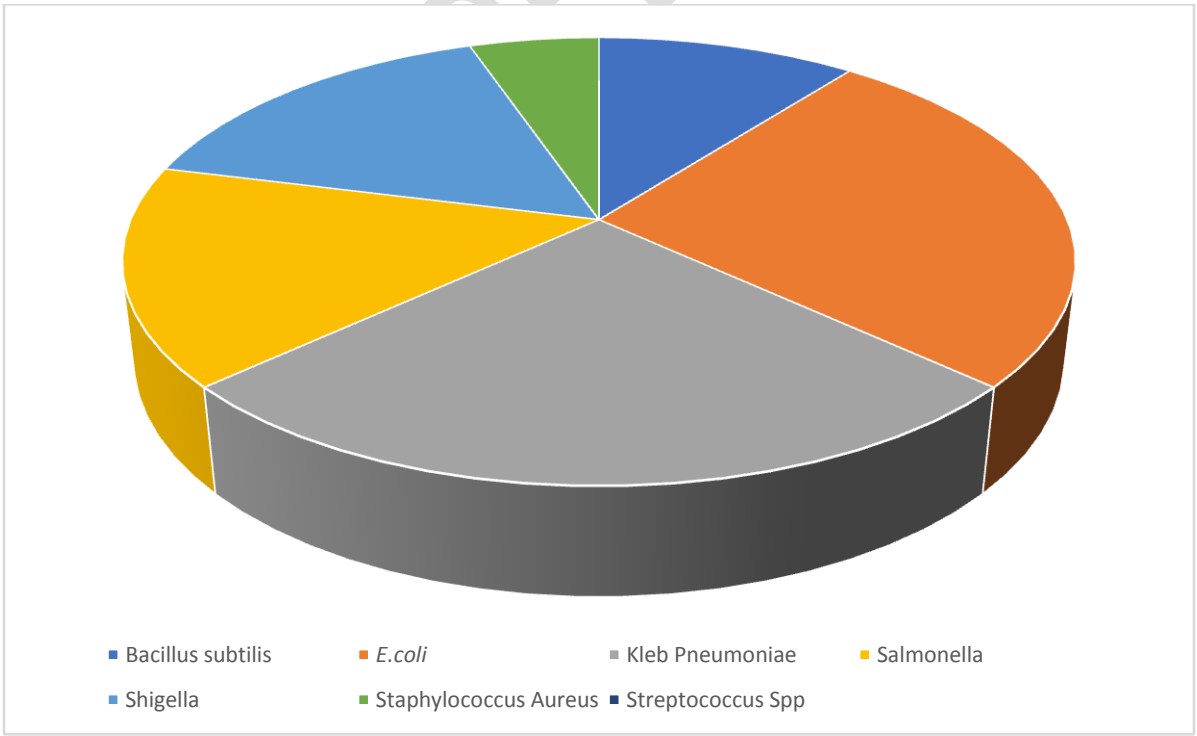

Figure 4: The isolated bacteria from the water sample at Local Government 3

Table 1: Rates of antimicrobial resistance among *E. coli* isolates

Classes of antibiotic	Name of antibiotic	*E. coli* (n = 19)		
		R	S	I
Cephalosporins (3rd Generation)	Cefotaxime25 µg	14	3	2
	Cefexime5 µg	13	3	3
	Ceftriaxone45 µg	14	5	0
	Cefuroxime30 µg	18	1	0
Cephalosporins (4th Generation)	Cefepime 30 µg	11	8	0
Penicillin derivatives	Ampiclox10 µg	18	1	0
	Augmentin 30 µg	19	0	0
Fluoroquinolones (1st Generation)	Nalidixic Acid 30 µg	19	0	0
Fluoroquinolones (2nd Generation)	Levofloxacin5 µg	12	6	1
	Ofloxacin 5 µg	11	8	0
Carbapenems	Imipenem10 µg	15	2	2
Nitrofuran	Nitrofuranton 300 µg	10	3	5
Amimoglycosides	Gentamicin 10 µg	13	6	0

S: SENSITIVE {Zone of inhibition ≥19mm}

R: RESISTANT {Zone of inhibition 14-18mm}

I: INTERMEDIATE {Zone of inhibition <13mm}

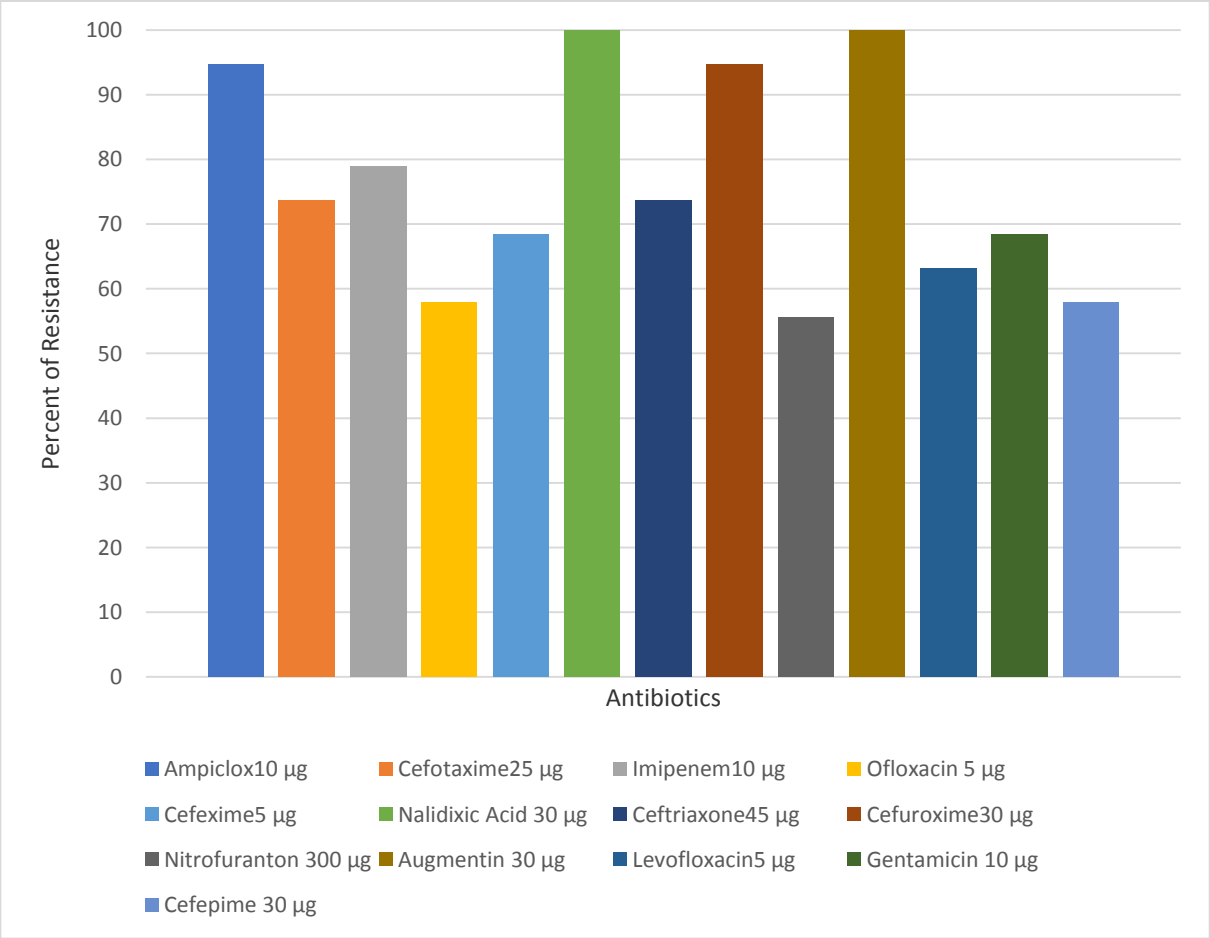

Figure 5: Antibiotic resistance pattern of all the 19 *E. coli* isolates against 13 different antibiotics

4.0 DISCUSSION

The presence, emergence, and spread of antibiotic resistance among bacterial pathogens due to contaminated water, pose a significant global health concern. In the pursuit of safeguarding public health and understanding the complexities surrounding antibiotic resistance, this research has explored the distribution of bacteria pathogens in surface and ground water in addition to antibiotic resistance patterns in *E. coli*[9].

The surface and ground water analysed for the presence of bacteria in all three local government area indicated *E. coli*(26.76%) and *K. pneumoniae* (21.13%) as the predominant bacteria isolates.The result of this study is in line with findings of previous research that reported a significant presence of *E. coli* in water samples.*E. coli* has been used as a measure of water contamination, especially contamination by feces[8]. The high percentage of *E. coli* isolated suggests sewage or fecal contamination of surface and groundwater in the three local

government areas. This is corroborated by the combined significant percentage (32.39%) of two common fecal pathogenic bacteria—*Salmonella* and *Shigella*—among the bacteria isolates. The possibility of water contamination by feces is substantial, considering the fact that about 48 million Nigerians still engage in open defecation according to the 2021 WASH NORM report. Hospital wastewater also serves as another channel for water contamination, when disposed inappropriately into natural water sources. The high percentage of*K.pneumoniae* isolated could be indicative of improper disposal of hospital waste, as *K. pneumoniae* is one of the prominent causes of nosocomial infections[10]. The implications of resistant pathogens in hospital wastewater makes the appropriate disposal of hospital waste necessary to avoid the spread of antimicrobial resistant bacteria—superbugs.

The resistance profiles observed in this study provide valuable insights into the antibiotic resistance patterns exhibited by these *E. coli* isolates. It is evident that all 19 (100%) *E. coli* strains demonstrated resistance to nalidixic acid—a first generation fluoroquinolones and augumentin—a pencillin derivative, highlighting the widespread resistance to these antibiotics within the sample population.Contrastingly, second generation fluoroquinoloneslevofloxacin (5µg) and ofloxacin (5 µg) had lower resistance percentages—63.16% and 57.89% respectively. The result of this study is in line with findings of previous research which reported 68.2% of 110 *E. coli* isolates showed fluoroquinolones resistance, showing significant resistance among the *E. coli* isolates[9]. Fluoroquinolones are used to treat illnesses that do not respond to other antimicrobial agents, according to the WHO, resistance in this class of antibiotics is of public health concern. Notably, penicillin-based antibiotics, such as augumentin (30 µg) and ampiclox (10 µg), which form a crucial part of contemporary medicine, exhibited very high resistance patterns. The enzymatic activity of resistant *E. coli* severely reduces their efficacy due to hydrolysis, these antibiotics are no longer effective against *E. coli*[11].

Futhermore, the third generation of cephalosporins e.g. cefotaxime (25 µg) at 73.68%, cefexime (5 µg) at 68.42%, ceftriaxone (45 µg) at 73.68%, and cefuroxime (30 µg) at 94.74%, exhibited substantially high antibiotics resistance pattern rates, emphasizing the challenges in using these antibiotics to treat infections caused by these*E. coli* strains. Conversely, the fourth-generation cephalosporin, cefepime (30 µg) displayed a relatively lower resistance rate (57.89%). The result of this study is in line with findings of previous research that reported that the lowest resistance of*E. coli* was seen against second-generation cephalosporins (26.66%) followed by fourth-generation cephalosporins (33.33%) and that the third and first generation cephalosporins showed 100% resistance against the antibiotics tested[12]. One fundamental factor contributing to the observed differences is the distinct molecular structure of these antibiotics, leading to variations in their spectrum of activity[13]. Additionally, fourth-generation cephalosporins like cefepime boast structural modifications that augment their effectiveness against resistant *E. coli* bacteria[14]. These structural disparities might render it more challenging for enzymes, produced by resistant *E. coli*, to confer resistance to cefepime when compared to the third-generation cephalosporins[14]. The resistance patterns observed in imipenem (78.95%) and gentamicin

(68.42%) in this study, contrasts with the findings of a study that reported low resistance rates in imipenem and gentamicin[15].

5.0 CONCLUSION AND RECOMMENDATION

In this study, distribution of pathogenic bacteria and patterns of antibiotic resistance of *E. coli* in residential water sources in a few regions of Oyo State, Nigeria were examined. The discovery of substantial pathogenic bacteria and antibiotic resistant*E. coli* in water samples brings attention to the possible health hazards linked to the transfer of antibiotic-resistant organisms from water used for domestic purpose to human population.It is crucial to put in place water treatment measures and quality monitoring programmes in order to guarantee that the populace has access to clean and safe drinking water. Antibiotic stewardship initiatives that encourage appropriate antibiotic use in the healthcare and agricultural sectors are also urgently needed. In order to prevent the spread of antibiotic resistance due to environmental pollution, it is important to reduce the unnecessary use of antibiotics in agriculture. Furthermore, it is crucial to continuously check water sources for the presence of resistant*E. coli* and other antibiotic-resistant bacteria. These initiatives may act as early warning systems, assisting in the detection of new risks to public health and directing focused remedies.Public awareness campaigns should be carried out to inform the public about the dangers posed by contaminated water. The need of adopting water treatment and hygienic practices as a way to protect the public's health should also be emphasised in these efforts.

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