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Plasmid Profile of Multi-Drug Resistant *Escherichia coli* and *Serratia marcescens* Isolated from Surface Water in Ado Ekiti, Ekiti State, Nigeria

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Abstract

This study examined the isolated bacteria from Elemi Stream in Ado Ekiti, Ekiti State, Nigeria for their microbial density, antibiotic resistance and plasmid profile. Pour plating was used to do the total bacterial and coliform counts, disc diffusion to evaluate the susceptibility of the isolated microorganisms, the alkaline lysis method to determine the plasmid profiles of the isolates, and agar gel electrophoresis to determine the coliform counts. The average total bacterial counts in sample A of the Elemi stream were 8.6 x 10^3 CFU/mL and the average total coliform counts were 8.4 x 10^3 CFU/mL, whereas the average total bacterial counts in sample B were 9.6 x 10^3 CFU/mL and 8.9 x 10^3 CFU/mL, respectively. The found isolates, together with their percentage distributions, include *Escherichia coli* (65.5%), *Enterobacter aerogenes* (13.8%), *Serratia marcescens*, and *Staphylococcus aureus* (10.3%). Augmentin demonstrated the highest level of resistance among the isolates, in contrast to Nitrofurantoin. Antibiotic resistance in various forms was discovered in *Serratia marcescens* and *Escherichia coli*. With molecular test, analysis revealed that the isolates with plasmid DNA had the gene encoding for antibiotic resistance, whereas the isolates without plasmids carried the gene encoding for antibiotic resistance on chromosomal DNA. The existence of multi-drug resistance plasmid-mediated bacteria in surface water is of great public health concern.

Keywords: Antibiotic resistance, Plasmid profile, Coliform, Multi-drug resistance

1. Introduction

The human body is made up of 90% water, which is vital to life and of fundamental importance to all living things (Williams *et al*., 2002). Water comes from two main sources: ground and surface sources. The availability of clean drinking water is a crucial component in preventing epidemic diseases and enhancing quality of life (Borchard *et al*., 2004). Water-borne diseases are widespread in developing nations where access to clean water and sanitary facilities are

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poor. The primary sources of water contamination are human sewage and parasites. Water pollution from harmful chemicals can also result from unintentional chemical pills, or land application of pesticides and fertilizers on fields, discharge from industrial sites, and sewage treatment facilities. Diverse water sources that have been contaminated can lead to illnesses including cholera, and even death. Two and a half billion people lack access to better sanitation, and more than 1.5 million children die from diarrheal infections every year (Admassu *et al*., 2004). According to the World Health Organization, unclean water is responsible for 80% of all ailments, with around one and a quarter billion people worldwide suffering from serious water related disease at any given time.

The prevalence of pathogens in drinking water distribution networks has long been a focus of research into the microbiological quality of drinking water (Berry *et al*., 2006). Pathogenic organisms present in water include bacteria such as coliform bacteria, *Staphylococcus aureus*, and specific pathogenic bacteria (such as *Vibrio cholerae*), viruses, and protozoan parasites such as *cryptosporidium* and *giardia*. Coliform bacteria are a common bacterial indicator of water contamination. Coliforms, which are typically not dangerous but are always present in human intestinal tracts and expelled with human waste in very huge numbers, are the most crucial microbiological indicator of drinking water quality. Since it is simpler to test for coliforms than specific types of pathogens, coliforms are utilized as indicator organisms for determining the biological quality of water. If coliforms are absent from the water, pathogens can be presumed to be absent as well. The likelihood of pathogens being present is thus reflected by the coliform count. Lead, arsenic, and nitrate/nitrite toxins can affect people and animals more quickly. Aquatic habitats are known to be a reservoir for antibiotic resistant bacteria and antibiotic resistant genes, making them one of the most urgent global public health issues (Baquero *et al*., 2008). Antibiotic resistance can spread through either acquired or natural (inherent) means. When an organism lacks an antibiotic transport system or an antibiotic molecule's target, as in the case of gram-negative bacteria, or when the cell wall is covered with an outer membrane that creates a permeability barrier against the antibiotic, bacteria may have inherent resistance to an antibiotic. Acquired resistance is the result of either horizontal or vertical gene transfer, which can take the form of conjugation, transformation, or transduction. Plasmids, which are tiny DNA molecules that can replicate independently of chromosome DNA within a cell, can also pass on antibiotic resistance genes to microbes. Through horizontal gene transfer, these plasmids are frequently transferred from one bacteria to another (even those of different species) (Kenneth, 2012).

Water is the most powerful resource on planet, not oil or minerals. It is the most prevalent substance on earth's surface, making up 90% of the weight of the human body and covering around 70% of the planet (William *et al*., 2002). Despite having no calories or organic nutrients, safe drinking water is crucial for both people and other living things, it plays a significant role in the global economy. Agriculture uses over 70% of the fresh water that people utilize (Baroni *et al*., 2007). In this context, the goal of the study is to assess the microbiological condition of the Elemi stream and conduct plasmid profile of isolates with various antibiotic resistance.

2. Materials and methods

2.1 Sampling, sterilization, media preparation

With the use of a sterile sampling vial, eight samples were taken. To prevent cross contamination, the sterile sampling bottle was lowered into the water 120 mm below the surface and immediately corked tightly. Within four hours of collection, the samples were delivered to the lab for quick analysis while being maintained in an ice-packed box.

To avoid contaminating the samples, all glassware was adequately sterilized. The glass items were sterilized in the hot air oven at 160 ℃ for two hours while the media were autoclaved at 121˚C for 15 minutes.

The media: *Salmonella Shigella* Agar, MacConkey Agar, Eosin Methylene Blue Agar, and Nutrient Agar were prepared as directed by the manufacturer. As a stock culture, nutrient agar slants were made in miniature vials.

2.2 Characterization and identification of isolates

2.2.1 Gram staining and motility test

To differentiate between gram-positive bacteria and gram-negative bacteria, gram staining, a differential staining technique was used.

2.2.2 Biochemical tests

Standard procedures were used for the Indole Test, Urease Test, Citrate Test, Methyl Red - Vogues Proskauer Test (MRVP), Methyl Red Test (MR), Vogues Proskauer Test (VP), Catalase Test, Oxidase Test, Coagulase Test, and Triple Sugar Iron (TSI).

2.3 Antimicrobial susceptibility test

Determination of antibiotic susceptibility of isolates was done by disc-diffusion method using Mueller-Hilton agar according to Clinical Laboratory Standards Institute, CLSI (2005). The agar was prepared according to manufacturer's instruction and the isolates were tested against

Ceftazidime (CAZ, 30µg), Cefuroxime (CRX, 30 µg), Gentamicin (GEN, 10 µg), Ofloxacin (OFL, 5 µg), Cefixime (CXM, 5 µg), Augmentin (AUG, 30 µg), Nitrofurantoin (NIT, 300 µg) and Ciprofloxacin (CPR, 5 µg).

The inoculums were standardized by adjusting its density to equal the turbidity of Barium sulphate, $BaSO_4$ (0.5 McFerland turbidity standard) and incubated at 35 ℃ for 18 hours. The diameter of zone of inhibition was measured to the nearest whole millimeter and interpreted using CLSI guideline (CLSI, 2005).

2.4 PLASMID ANALYSIS

2.4.1 Plasmid Extraction

To pellet cells, 1.5 mL of overnight culture was centrifuged for 1 minute in a micro centrifuge. Following that, carefully decant supernatant leaving 50-100 µL with cell pellet and vortex at high speed to completely re-suspend cells. Then 300 µL of Tris-HCl, EDTA and NaCl (TENS) was added. An inverted tube was used to stir the material 3-5 times until it became sticky. The preparation was then given 150 µL of 3.0M sodium acetate, pH 2 and vortexed to thoroughly mix it. The solution was spun in a micro centrifuge for 5 minutes to pellet cell debris and chromosomal DNA, and the supernatant was transferred to a new tube and well mixed with 900 µL of ice cold 100% ethanol. The pellet was cleaned twice with 1 mL of 70% ethanol and dried after being spun for 10 minutes to pellet plasmid DNA (white pellet was detected). For future use, the pellet was re-suspended in 20-40 µL of TENS buffer.

2.4.2 Agarose gel electrophoresis

Agarose powder (for plasmid DNA) weighing 0.8 g was added to 150 mL of 1X Tris Borate EDTA (TBE) buffer, it was dissolved by boiling with the aid of a magnetic stirrer. The mixture was then gently swirled while 10 µL of ethidium bromide was added when it had cooled. The loading dye was then combined with 15 µL of the sample. After that, it was added to the electrophoresis tank filled with 1X TBE buffer with the comb and bubbles were avoided Following the meticulous loading of samples into the comb's wells, lane one was loaded with markers, then the controls. The electrophoresis was then carried out at 60–100 V and a UV-transilluminator was used to view the gel.

2.5 Physico-chemical and mineral analysis of water

A and B-labeled water samples were examined. A KENT EIL 7020 (Kent Industrial Measurement Limited, Surrey, England) was used to monitor the pH and turbidity. After standardization with Potassium Chloride (KCl) solution, conductivity was measured with a CDM83 conductivity meter (By Radiometer A/S Copenhagen, Denmark) and other parameters

were determined using various standard methods. Total hardness was assessed by EDTA titration using Erio-chrome Black-T-indicator, whereas total alkalinity was determined by titration using phenolphthalein and methyl orange indicator (AOAC, 2005).

3. RESULTS

Table 1 displays the mean total bacterial counts and mean total coliform counts of the water samples at various times of collection. While sample B's mean values are 9.6 x 10^3 and 8.9 x 10^3 CFU/mL, sample A's mean total bacteria counts (TBC) and mean total coliform counts (TCC) are 8.6 x 10^3 and 8.4 x 10^3 CFU/mL, respectively.

Table 1: Bacterial density of water samples

SAMPLES	Total Bacterial Count (TBC) CFU/mL	Total Coliform Count (TCC) CFU/mL
A1	2.2 x10^3	2.0 x10^3
A2	1.9x10^3	2.4 x10^3
A3	2.4 x10^3	1.8 x10^3
A4	2.1 x10^3	2.2 x10^3
Mean value	8.6 x10^3	8.4 x10^3
Range	1.9x10^3 – 2.4x10^3	1.8x10^3 – 2.4x10^3
B1	2.6 x10^3	2.1 x10^3
B2	2.3 x10^3	2.4 x10^3
B3	1.9 x10^3	2.5 x10^3
B4	2.8 x10^3	1.9 x10^3
Mean value	9.6 x10^3	8.9 x10^3
Range	1.9 x10^3 - 2.8 x10^3	1.9 x10^3 - 2.5 x10^3

Table 2: Percentage distribution of isolates from Elemi stream in Ekiti State

Organisms	Isolated numbers	Percentage of occurrence
Escherichia coli	19	65.5%
Enterobacter aerogenes	4	13.8%
Staphylococcus aureus	3	10.3%
Serratia marcescens	3	10.3%
Total	29	100%

Table 3: Antibiotic susceptibility test of Bacteria isolated from the water

S/N	ISOLATES	CPR (5µg)	CAZ (30µg)	CRX (30µg)	GEN (10µg)	CXM (5µg)	OFL (5µg)	AUG (30µg)	NIT (300µg)
1	*Escherichia coli*	S	R	R	S	I	S	R	R
2	*Escherichia coli*	R	R	R	R	I	R	R	R
3	*Staphylococcus aureus*	S	I	R	R	R	S	R	S
4	*Enterobacter aerogenes*	R	R	I	I	R	R	R	S
5	*Escherichia coli*	R	R	I	I	R	S	R	R
6	*Escherichia coli*	R	R	R	R	R	I	R	I
7	*Escherichia coli*	R	R	R	I	R	R	R	R
8	*Staphylococcus aureus*	R	R	R	R	R	R	R	I
9	*Escherichia coli*	S	S	S	S	S	S	I	S

R-Resistant, S-Susceptible, I-Intermediate

Figure 1 shows the plasmid profile of isolates from Elemi Stream. A total of Eight isolates with multiple antibiotic resistance pattern were selected for plasmid analysis using gel electrophoresis. Six of the isolates harbored 23.13 kilobase pairs (Kbp) sized plasmid while two isolates did not harbor plasmid.

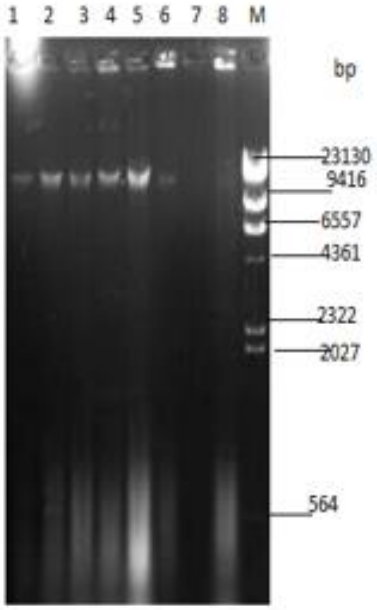

Figure 1: The plasmid profile of bacteria isolates with multiple antibiotic resistance

The physico-chemical and mineral analysis of the water samples was presented in Table 4

Table 4: Physico-chemical and Minerial Analysis of Water Samples

Parameter	Result	
	Sample A	Sample B
Temperature (^{0}C)	26.20	26.20
Turbidity (NTU)	0.01	0.01
Conductivity (μhoms/cm)	$1.2x10^2$	$1.5x10^2$
pH	7.40	7.30
Total dissolved solid (mg/L)	100.28	100.03
Total solid (mg/L)	118.36	119.65
Total suspended solid (mg/L)	18.08	19.62
Total alkalinity (mg/L)	40.25	35.40
Acidity as $CaCO_3$ (mg/L)	4.60	4.70
Total hardness (mg/L)	123.00	122.50
Chloride (mg/L)	20.33	20.30
Sulphate (mg/L)	23.50	24.20
Phosphate (mg/L)	13.50	14.00
Nitrite (mg/L)	12.49	12.50
DO (mg/L)	9.50	8.50
BOD (mg/L)	4.50	3

DO= Dissolved Oxygen, BOD= Biochemical Oxygen Demand, ND = Not Detected

4. DISCUSSION

The availability of clean drinking water is a crucial component in preventing epidemic disease and enhancing quality of life (Borchard *et al*., 2004). One of the primary causes of sickness in Nigeria and throughout the world has been linked to the drinking of contaminated water. This is because the degradation of natural water quality has increased due to an increase in human population and urbanization. While water is necessary for life, it also serves as a home for microorganisms and can act as a reservoir for viruses that could infect individuals who consume it.

The inherent risks connected with consuming this water in its untreated condition are brought to light by this research, which emphasizes the necessity for public education or knowledge of these risks. The results of the microbiological and physicochemical tests performed on samples taken from the Elemi stream reflect the rate of contamination, particularly contamination brought on by human activities in the streams, and thus cast doubt on the portability, quality, and suitability of the water for drinking and other domestic uses.

All of the water samples had a total bacterial count that varied greatly and was higher than the recommended limit for drinking water (WHO, 2003). All of the water samples had a total coliform count that was much greater than the Maximum Contamination Level (MCL) for coliform, which is defined as a total coliform level of zero per 100 milliliters of water (EPA, 2003). According to Osuinde and Enezie (1999), this higher coliform count is a sign that the water sources have been faecally contaminated by either human or animal origin. In line with Banwo (2006) assessment, which claimed that the presence of bushes and shrubs likely makes it feasible for animals to be aware of the source of water and subsequently pass their faeces into the water bodies.

The results of the current investigation showed that all of the water samples examined were of poor quality, as per the standards for drinking water quality. This showed extremely high levels of isolated microbe antibiotic resistance as well as pollution of the Elemi stream. Environmental contamination linked to human activities, such as sewage disposal and trash dumping, has contaminated and jeopardized Elemi stream. People who use and consume the water may contract diseases and experience bad health as a result of the presence of such microorganisms.

The isolation of dangerous organisms like *Escherichia coli* and *Enterobacter aerogenes*, which have been linked to gastrointestinal diseases like dysentery, diarrhea, typhoid fever, and more, is important for public health (EPA, 2003). The overwhelming presence of *Escherichia coli* in all of the water samples and the consequent non-portability of the water due to this organism's

production of enterotoxin are both extremely significant indicators of faecal contamination of the water (Faria *et al*., 2009). Even while the majority of *Escherichia coli* strains are not harmful, their presence suggests that potentially harmful organisms may also be present. According to Wanke *et al*., 1990, water is deemed safe when it is clear of *Escherichia coli*.

The bulk of the gram-negative bacteria that were recovered in this investigation were found to be resistant to more than three drugs. Antibiotic resistance is a global public health issue that is still getting worse. Bacteria and other organisms resistant to antibiotics have become resistant to several medications. When exposed to antibiotics, some bacteria develop living walls, which operate as a physical barrier to protect them and worsen the problem of drug-resistant illness (Matic *et al*., 1996). The increased rate of irrational antibiotic consumption, the spread of resistant isolates among people, the consumption of food from animals that have received antibiotics, self-medication and non-compliance with medication, and the sales of substandard drugs may all be contributing factors to the high levels of antibiotic resistance seen in this study compared to other studies.

They were resistant to three (3) to seven (7) different types of antibiotics. The three drugs Cefixime, Augmentin, and Ceftazidime showed the highest rates of overall resistance, which is consistent with the findings of Toro-Olu and Dincer (2008), who found the same multi-drug resistance in isolates obtained from ground water. The findings of this study were consistent with those of Lin *et al*. (2004), who characterized 113 enteric bacteria from the Mhlathuze River in South Africa, including *Escherichia coli*, *Citrobacter freundii*, *Enterobacter spp*., and *Serratia marcescens*. They discovered that 94.7% of these bacteria were resistant to more than two antibiotics, and 75.2% of the isolates were multidrug resistant.

Eight (8) multi-drug resistant cells from the Elemi stream were examined for plasmid in this investigation. Six of the isolates included plasmids, whereas the other two did not, but both were antibiotic-resistant. One method of determining if the gene encoding antibiotic resistance is found on chromosomal DNA or plasmid DNA is plasmid analysis. Plasmid-mediated multi-drug resistance (MDR) and the detection of high molecular weight (23.13 Kbp) plasmids in the isolates were both present. (Mcpherson and Grealt, 1986) demonstrated that a number of enteric bacterial strains with high molecular weight plasmids and antibiotic resistance may pass on their resistance to recipient bacteria. Given the enormous potential for infection, the spread of resistance (R) genes and plasmids poses a threat to public health.

One plasmid was present in each of the isolates examined for this study. Once an R-plasmid is present in a bacterial cell, Willey *et al*. (2008) claim that it can be quickly transferred to other cells by regular gene exchange procedures like conjugation, transduction, and transformation.

A single plasmid harboring a gene for resistance to numerous drugs may result from this gene exchange. The quantity and molecular weights of the plasmids were used to generate the plasmid pattern. Plasmids are one of the most significant mediators promoting the rapid spread of antibiotic resistance among bacteria, as is well known (Dale and Park, 2004).

After physico-chemical analysis of water samples, the pH range of the water samples is somewhat consistent with Imevbori (1985) claim that the pH of water bodies ranged from 6.5 to 7.4. If the water is properly handled and treated, the pH number is a sign of the water's portability. According to Banwo (2006), the temperature soared from 280 ℃ to 300 ℃ on relatively hot days, which is thought to have contributed to the temperature range. The observed turbidity was still close to the EPA threshold, which also indicated that the total suspended solid (TSS) value was low. Asano (2007) defined turbidity as the cloudiness of a liquid caused by the presence of suspended particles and emphasized how the presence of suspended solids protects bacteria. All of the water samples' total dissolved solid counts met the EPA (2002) requirement.

5. CONCLUSION

Increased faecal coliform levels is signaled by a problem with water treatment, a breach in the distribution system's integrity, and potential pathogen contamination. The study area is primarily polluted by faecal contamination; sewage leakage, improper organic waste disposal, and illegal refuse dumping all have a direct impact on the water that is used for drinking and other domestic purposes in this area. It is crucial to underline that these streams are home to a variety of harmful bacteria with diverse antibiotic resistances. The community's residents face a major health risk due to the presence of bacteria, particularly intestinal bacteria that are resistant to antibiotics. These organisms can operate as a source of genes that make bacteria resistant to antibiotics, which could then spread to potentially harmful bacteria in the ecosystem. Before the streams are used for human consumption, they must undergo routine examination to see if the coliform level is within acceptable bounds and to see if the level of metals and mineral composition is also within acceptable bounds. Waste water should not be dumped on surrounding soils, and soil erosion should be properly controlled. The findings of this study challenged scientists to continue developing new antibiotics in order to treat diseases brought on by these resistance strains as well as the need for ongoing pollution monitoring programs of Nigeria's surface water.

Conflict of interest: None

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