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Original Research Article

Sensitivity Pattern of Some Commercial Antibiotics to Bacteria Isolated from Cotton Leaf Treated Well Water

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ABSTRACT

Water pollution occurs when harmful substances or microorganisms contaminate water bodies such as Rivers, Streams, Wells, and Lakes thereby reducing the quality of water and rendering it unfit for use in the environment or by humans. In this study, water samples were collected from a well five times and analyzed to determine the efficacy of commercial antibiotics on bacteria isolates obtained. A fifth sample was used as the control. Antimicrobial activities were observed against five (5) isolated bacteria: Escherichia coli, Klebsiella pneumonia, Shigella dysenteriae, Salmonella typhimurium and Proteus vulgaris. Physicochemical analysis, bacteriological analysis, biochemical tests and morphological identification of isolates were also carried out. From the results, the highest pH value of 6.9 was observed in the control, while the fourth sampling time had the lowest pH value of 3.5. Turbidity was highest; 0.564 NTU in the fourth sample with a contact time of 0 hrs and it was lowest (0.030 NTU) in the control sample with a contact time of 72 hrs. Total bacterial counts showed 105 × 10 ^{4}cfu/ml as the highest value for bacteriological analysis while 2 × 10^{4} cfu/ml was the lowest value from both total coliform counts and total bacterial count in the bacteriological analysis. The highest inhibition zone of 30 mm was observed in Escherichia coli and Salmonella typhimurium on Amoxicillin and Chloramphenicol respectively while Chloramphenicol showed the lowest significant action with an inhibition value of 18 mm observed in Klebsiella pneumonia. Results of this study indicated the sensitivity pattern of some commercial antibiotics on isolates from well water.

1. INTRODUCTION

Water is a major need on the earth. It is the largest occurring medium on earth, the main fluid constituent of most living organisms and also essential for all forms of life. It is essential for survival, health, hygiene, agriculture and industrial processes. Water is a polar inorganic compound that is characterized as a tasteless,

colorless and odorless liquid thus, it is described as the "universal solvent" (U.S. Geological Survey, 2017). Biologically, water has many distinguishing properties that are critical for life.

Water is a medium which can harbor thousands of microorganisms some of which are disease causing pathogens e.g. bacteria, viruses, protozoa etc. From a sanitary standpoint, water is classified as potable, contaminated or polluted (Fawell and Nieuwenhuijsen, 2003). Pollution of water occurs from the misuse of water usually as a result of human activities leading to the contamination of water bodies (lakes, rivers, groundwater etc) (West, 2006). WHO (2006) states that countless lives are lost annually due to drinking and use of contaminated water. The quality of natural water bodies is on a rapid decline due to activities and discharges arising from population growth, urbanization and industrialization (Anyanwu and Ukaegbu, 2019). However, water is a unique substance because it has self-purification abilities and contaminants are removed during biological processes. Pollution of water has become widespread thus, posing a great risk to human health. Unsafe water kills people annually (Inyinbor, 2018). Underground water is one of the least visible but most important natural resources. Pollution of groundwater occurs when contaminants from pesticides, fertilizers, landfill wastes, leachate from sewers percolate through aquifers making the water unsafe. Once groundwater is polluted, it can be rendered unusable for decades or years (Denchak, 2018).

A wide range of diseases result from the contamination of drinking water from human or animal origin (Prescott *et al.*, 2005; Dzwairo *et al.*, 2006). Waterborne diseases can thus be transmitted through faecal oral routes (Chess, 2000). Water-borne pathogens are a major cause of illness from contaminated drinking water. Diseases spread by contaminated water include Cholera, Diarrhea, Typhoid, Legionnaires' disease etc. (Denchak, 2018). To prevent the transmission of pathogens through polluted water, appropriate treatment of water is essential. Conventional water treatment processes for potable water include the use of inorganic, synthetic organic polymer and naturally occurring coagulants (Okuda *et al.*, 2001). The high cost of importing water treatment chemicals like Aluminium Sulphate (Alum – a common coagulant) coupled with the carcinogenic nature of its residues have made the use of untreated water from boreholes/shallow wells an alternative (Litherland, 1995).

Resistance of microorganisms to available antimicrobial agents has resulted in harmful effect to human health over the years (Prestinaci *et al.*, 2015). The increase in antimicrobial resistance of pathogenic microorganisms particularly in water is a major concern to the society (Aderanti *et al.*, 2019). Antibiotic sensitivity is the susceptibility of bacteria to antibiotics. Antimicrobial testing is used to determine the potential effectiveness of specific antibiotics on microorganisms. It is also used to evaluate the resistance of microbes to certain antibiotics. Bacteria and fungi have the ability to develop resistance to antibiotics and antifungal drugs at any time (American Association for Clinical Chemistry, 2018).

The aim of treatment of drinking water is to remove impurities and bacteria in order to meet the quality guidelines of drinking water (WHO, 2004). Thus, the aim of this study is to determine the sensitivity pattern of some commercial antibiotics on isolates from well water.

2. MATERIALS AND METHODS

2.1. Source of Water Samples

The experiment involved the collection of well water samples from the same well over a period of time including the control using sterile plastic sample containers. Samples were collected from a well located at Gargo Area of Tanke, Ilorin, Kwara State, Nigeria. One liter of samples was put in each of five (5) sample containers.

2.2. Ethanolic Extraction of Plant Extracts

Forty-eight gram of cotton leaves powder were soaked in 350 mL of 70% ethanol for 4 days. The extract was filtered and distilled using a quick fit condenser. After distillation, a dark brown liquid extract and the original solvent was obtained. The liquid extract was further concentrated by placing the beaker over a water bath after which a thick gummy liquid was obtained. Four dilutions were prepared and used. They include 0.12% (sample 1), 0.20% (sample 2), 0.25% (sample 3) and 1% (sample 4). The control was well water that was not treated with cotton leaf.

2.3. pH and Turbidity Measurement

The pH and turbidity were measured according to standard methods described by Fawole and Oso (2007)

2.4. Antibacterial Bioassay

The culture media used for bacterial count, isolation, sub culturing, biochemical characterization and antibiotic sensitivity were Nutrient Agar, Eosin Methylene Blue Agar, Salmonella-Shigella Agar, Simmon's Citrate Agar. Preparation of media was carried out in accordance with the directions provided by the manufacturer. All media along with Petri dishes, pipettes, glassware were sterilized in an autoclave at 121 ^{o}C for 15 mins. Pouring of media into plates as well as other microbiological processes was carried out aseptically.

2.5. Bacterial Characterization and Identification

The isolates were characterized based on Gram staining, physicochemical analysis such as pH and turbidity, morphological features such as shape, pigmentation, optical character and colony surface. Also, biochemical tests such as Catalase test, Oxidase test, Indole test were carried out according to the protocol described by Fawole and Oso, (2007). Pure cultures of bacterial isolates were identified on the basis of their colonial morphology, cellular morphology and biochemical characteristics according to the scheme of Cowan and Steel (Barrow and Feltham, 1995)

2.6. Antibiotic Sensitivity Test

This was determined using the Kirby Bauer comparative disc diffusion method of known antibiotic disc concentrations (Hudzicki, 2009). The antibiotic discs used were all gram negative with a diameter of 8 mm. Molten nutrient agar was poured into a sterile Petri dish and swirled. The plates were allowed to solidify after which the isolates from the pure culture obtained were streaked on the plates. The antibiotic discs were placed on the plates and the plates incubated at 37 ^{o}C for 24 hrs. After 24 hrs, the plates were examined for zones of inhibition and the diameters measured (CLSI, 2006).

3. RESULTS AND DISCUSSION

Results obtained from this work indicated certain important values for control sample and the four active test samples. Figure 1 compares the pH of both the control and the treatment samples. The control was well water that was not treated with cotton leaf). The pH of the control was generally higher than that the other samples. pH decreased as the time increased. Figure 2 is a comparison of the turbidity values of all the sampled well water. The values obtained from analysis of total suspended solids (TSS) is indicated in Figure 3. The occurrence of coliform, bacteria and *Salmonella-Shigella* counts in the control and treatment samples are shown on Table 1. Organisms isolated from the samples were *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Salmonella thypimurium* and *Shigella dysentriae*. Similar bacteria were isolated from water samples by Kolawole *et al.* (2013). Total bacterial count was higher than total coliform count

while *Salmonella-Shigella* count was higher than total coliform count. Table 2 describes the morphological characteristics of the organisms and reactions to various biochemical tests. Table 3 shows the measurement of zones of inhibition of the isolates.

High concentrations of suspended solids (TSS) prevent sufficient oxygen circulation in water thus leading to the death of organisms in the water. The physicochemical properties of the water sample had variations in pH to range between 3.5 - 6.9. The normal pH range for groundwater system is between 6.0 - 8.5 (Ravi *et al*., 2016). The pH result obtained in this study shows a near acidic nature of the well water sampled and this is likely attributable to dissolved minerals that are acidic in the surrounding soils especially near the aquifers. Prescott *et al*. (2005) also states that external pH might alter the ionization of nutrient molecules thus, reduce their availability to the organisms. The pH of water plays an important role in the survival rate of organisms (Fawole and Oso, 2007).

Human activities that disturb land e.g. construction, agriculture, mining, etc, can lead to high level sedimentation around the waste bodies when runoffs from rainfall occurs. In drinking water, the higher the turbidity level, the higher the risk of gastrointestinal diseases (Mann *et al*., 2007). According to USEPA (1999), the amount of turbidity that can be effectively removed depends on the technology used in treatment. Permissible limits are set by governments or organizations on the allowable turbidity in drinking water. The European Standards permits a turbidity level of not more than 4 NTU while the World Health Organization permits a turbidity level of not more than 5 NTU but ideally, it should be below 1 NTU.

The main source of pathogens in drinking water results from faecal or animal origin occurring as a result of improperly treated septic and sewage discharges, leaching of animal manure and storm water runoff. Coliforms are group of bacteria commonly found in the environment. They are usually not pathogenic but their presence indicates the degree of contamination of water supply by more harmful microorganisms as well as the sanitary quality of water (Khvaschevskaya *et al*., 2016). The maximum acceptable concentration for drinking water should be non-detectable (ND) per 100 ml. This means in order to conform to the guideline, for every 100 ml of drinking water tested, no coliforms or *E. coli* should be detected. The result obtained in the bacteriological analysis, shows a high value in total coliform counts as against the acceptable standard and is indicative of faecal pollution of water. This may be due to proximity of the well to a septic tank, exposure of the well (if not covered) to organisms in air, lack of proper sanitary hygiene. In addition, based on the morphological and biochemical tests, the presence of the bacterial species could be as a result of human activities, the use of dirty buckets which may carry soil.

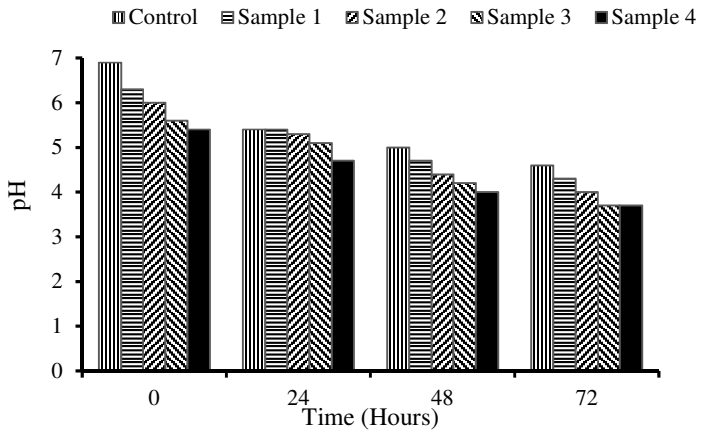

Figure 1: pH values of the well water samples

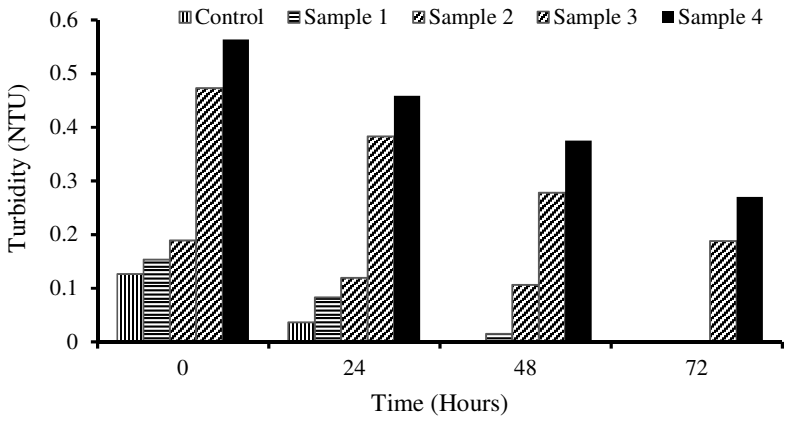

Figure 2: Turbidity values (NTU) for the well water samples

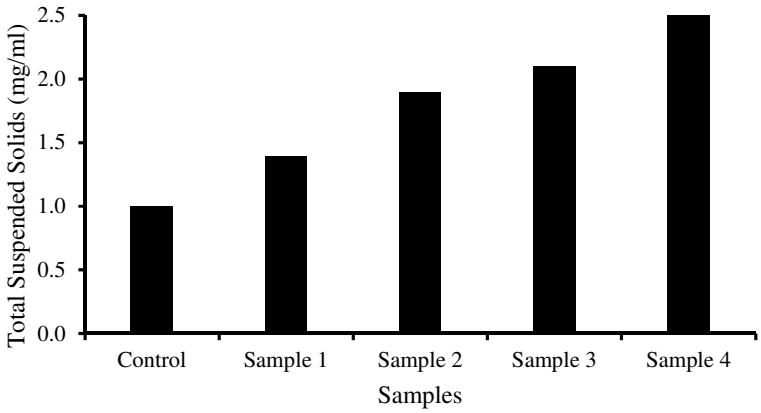

Figure 3: Total suspended solids (TSS) in the water samples

Antibiotics are extremely important to medicine. Although, there has been a recent emergence of antibiotic-resistant bacteria due to chromosomal mutations which has reduced the ability to treat bacterial infections (Sommer *et al.*, 2017; Barlow, 2018). Antibiotic resistance is a major constraint to global public health. The discovery of new antibiotics is slow and to prevent bacterial infections as well as ensure proper treatment, new antimicrobial strategies are required (Carola *et al.*, 2019). Minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobial agents that prevent visible growth of a microorganism under known conditions. Results obtained from antibiotic sensitivity test showed that all microorganisms isolated were resistant to Septrin (SXT) and Streptomycin (S). The variation in data obtained using MIC assay may be influenced by factors such as the inoculums size, type of growth medium and the incubation time (Balouiri *et al.*, 2016). The use of disc diffusion in this study is due to its simplicity and capacity to analyze large number of test samples.

Table 1: Bacteriological analysis

Sampling period (hours)	No. of Counts (cfu/ml x 10^4)					Analysis
	Wc	W_1	W_2	W_3	W_4	
0	30	20	14	10	7	Total coliform count
24	34	19	11	8	5	
48	39	15	9	6	4	
72	40	12	7	4	2	
0	80	74	46	29	5	Total bacterial count
24	88	69	40	17	4	
48	94	61	30	10	3	
72	105	55	27	6	2	
0	36	28	21	18	9	*Salmonella-Shigella* count
24	38	25	17	12	6	
48	42	22	13	8	4	
72	47	19	10	6	3	

Wc = Control, W_1 - W_4 = test sampling sites

Table 2: Morphological characteristics and biochemical tests

Isolate	Shape	Pigmentation	Colony Surface	Optical Character	Consistency	Gram Stain	Glucose	Lactose	Oxidase	Indole	Catalase	Urease	Citrate Utilization	Lysine Decarboxylase	Motility	βgalactosidase	Probable Organisms
i.	Circular	Greenish metallic sheen	Smooth	Translucent	Mucoid		AG	AG		+	+		+	+	+		*Escherichia coli*
ii.	Irregular (swarming colonies)	Cream	Rough	Translucent	Granular		A			+	+	+	+		+		*Proteus vulgaris*
iii.	Circular	Pink	Rough	Opaque	Mucoid		AG	AG		+	+	+	+	+		+	*Klebsiella pneumoniae*
iv.	Circular	Pink	Smooth	Opaque	Mucoid		AG				+		+	+			*Salmonella tyhimurium*
v	Circular	Cream	Smooth	Transparent	Mucoid		A				+						*Shigella dysentriae*

Table 3: Measurement of zones of inhibition of isolates

Isolates	Inhibition (mm)									
	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
Klebsiella pneumonia	0	18	0	0	30	21	0	0	24	0
Escherichia coli	0	19	0	0	0	27	25	20	23	0
Shigella dysenteriae	0	29	26	21	0	0	20	0	0	0
Salmonella typhimurium	0	30	22	28	0	0	27	0	0	0
Proteus vulgaris	0	0	26	24	0	0	25	26	29	0

Sensitive: 18-30 mm, Resistant: < 18 mm, SXT = Septrin (30 µg), CH =Chloramphenicol (30 µg), SP =Sparfloxacin (10µg), CPX = Ciproflaxacin (10µg), AM = Amoxacillin (30 µg), AU =Augmentin (30µg), CN =Gentamycin (10µg), PEF =Pefloxacin (30 µg), OFX =Tarivid (10µg), S=Streptomycin (30µg)

Deep waterbodies are generally pure having most of their contaminants filtered off in their downward passage (WHO, 2006). According to Sheenam *et al.* (2018), water quality management is a great task that should be controlled by monitoring of waste disposal, untreated discharge or effluent.

4. CONCLUSION

Based on the isolated microbes, this study is a pointer to the fact that contamination of well water is a major concern. It is thus necessary for water bodies to be adequately maintained and protected against all forms of anthropogenic activities and pollution from animals thereby improving the quality of water.

5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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