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Screening for antibiotic resistant bacteria from two water streams in Iwo town

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Abstract

The sensitivity pattern of antibiotic resistant bacteria from Oke-odo stream and Aiba stream were investigated. *Escherichia, Klebsiella, Citrobacter, Serratia, Enterobacter, Salmonella, Staphylococcus and Proteus* were isolated. The Organisms were Gram-negative. All the organisms were 100% resistant to septrin. Almost all the organisms were resistant to amoxicillin and augmentin. The most dominant bacteria was *Citrobacter*, followed by *Escherichia* and *Serratia.* Antibiotic sensitivity was carried out using antibiotic sensitivity discs containing 10 antibiotics. *Samonella, Enterobacter* and *Klebsiella* were 100% resistant to septrin, amixacillin, and streptomycin.*Serratia*was 100% resistant to septrin, sparfloxacin and amixacillin and 50% resistant to augmentin, gentamycin, perfloxacin and streptomycin. *Escherichia was* mostly 66.66% resistant to all the antibiotics, except septrin.

Keywords: Sensitivity, Antibiotic, Stream

Introduction

Water has always been an important life-sustaining liquid to humans and it is essential to the survival of all known organisms (1) Every effort should be made to achieve a drinking water as safe as possible (2).Water is a chemical compound with the chemical formula H_2O. Good quality water is odourless, colourless, tasteless, and free from faecal pollution (3). Water is a liquid at standard ambient temperature and pressure, but it often co-exist on earth with solid state, ice and gaseous states (water vapour or steam) (4).

Water is very important to man in the following ways: for keeping our environment clean, drinking, irrigation purposes, washing, bathing, recreational purposes such as swimming, spa, e.t.c. (5). Despite the fact that water has been known for its medical value, it also has the possibility of carrying diseases. Waterborne diseases don't just appear like that. Typhoid fever or cholera does not develop as a result of the filth in water. It comes from a person with these diseases or from a carrier of these organisms (6). Waterborne diseases continue to be one of the major health problems especially in developing countries. The high prevalence of diseases such as diarrhoea, typhoid fever, cholera and bacillary dysentery among the populace has been traced to the consumption of unsafe water and unhygienic drinking water production practices (7). The most dangerous form of water pollution occurs when faecal contaminants enter the water supply. Pathogens such as *Salmonella* spp., *Shigella* spp., *Vibrio cholera* and *Escherichia coli* being shed in human and animal faeces ultimately find

their way into water supply through seepage of improperly treated sewage into groundwater (8).

Antibiotic Resistanceis a form of drug resistance whereby some (or, less commonly, all) sub-populations of a microorganism, usually a bacterial species, are able to survive after exposure to one or more antibiotics; pathogens resistant to multiple antibiotics are considered multidrug resistant (MDR) or, more colloquially, superbugs (9).The driving force of antibiotic resistance is the indiscriminate widespread use of antibacterial drugs (10). In human medicine, the major problem of the emergence of resistant bacteria is due to misuse and overuse of antibiotics by doctors as well as patients. There are various factors that contribute to the occurrence of resistance such as commercial promotion; use of monotherapy, prescriber's prescriptions, over the counter sale of antibiotics, under use of microbiological testing and globalization (11). To date, the majority of the studies of resistance patterns have focused on pathogenic bacterial populations, but it is known that commensal bacteria are also common reservoirs of antibiotic resistant genes (12).Some of the antibiotic resistant pathogens that have been identified are; *Staphylococcus aureus, Campylobacter , Streptococcus pyogenes, Pseudomonas aeruginosa, Clostridium difficile, Salmonella, Escherichia coli,* and *Mycobacterium tuberculosis.*

Screening for antibiotic resistant bacteria in natural ecosystems is highly relevant and worldwide studies have been carried out to identify environmental reservoirs of bacterial antibiotic-resistance in wild-animal populations and natural water supplies (13). With the topic being broken down, we would be able to identify these environmental reservoirs of antibiotic-resistant bacteria.

Materials and Methods

Sampling:

Water samples were collected using sterile plastic bottles from two (2) streams in Iwo and labelled A and B. The samples were taken on a fortnight basis and were immediately brought to the laboratory for analysis within 24 hours. The samples were analysed for ph and turbidity. The bacterial isolates were identified and tested for antimicrobial sensitivity.

Biochemical Tests:

a. pH: The pH meter was first dipped into sterile de-ionized water, which should read a pH of 7.0. Thereafter, it was dipped into the universal bottle which reads the content (stream water). Samples that had a pH less than 7 were neutral and those that had a pH above 7 were said to be alkaline.

b. Turbidity: A cuvette containing sterile de-ionised water as control was first inserted into the machine to standardize the machine to zero value. The sample from the stream was then poured into the cuvette and inserted into the colorimeter. It was ensured that the water did not exceed the marked portion of the cuvette. The colorimeter was closed and the reading was taken at 600nm using the absorbent option.

c. Biochemical Oxygen Demand (BOD): It was done to determine the amount of oxygen present in each sample. A high BOD value shows the level of contamination of the sample.

Bacterial counts:

According to the methods described by (21), nine millilitres of distilled water was dispensed into test tubes with a pipette. The tubes were covered with cotton wool, plugged tightly and autoclaved at 121^{o}C for 15 minutes. After autoclaving, one millilitre of the water samples each were inoculated into the first test tube and the water was serially diluted five times. 10 fold dilutions were carried out for each sample, i.e. 10^{-1} to 10^{-6}.

One millilitre of the sample from the 10^{-6} test tube was pipette aseptically into a sterile Petridish. Nutrient agar was poured over the sample and mixed by swirling gently on the plate. The agar was allowed to set and was incubated at 37^{o}C for 24 hours and colonies were counted (cfu/ml).

Preparation of media:

The media used was Nutrient Agar. The preparation and appropriate composition was done according to the manufacturer specification. The media was prepared with distilled water and the media was autoclaved at 121^{o}C for 15 minutes. Al these processes were carried out aseptically. After 15 minutes, the media in the autoclave was allowed to cool to about 45^{o}C. It was then poured aseptically into sterile Petri-

dishes and allowed to set. For the preparation of nutrient agar slants, the composition and the preparation were done according to the manufacturer's instruction and it was heated in a beaker using a stirrer and heater. The heated mixture was dispensed into Maccartney bottles and it was autoclaved at 121^{o}C for 15 minutes. The Macartney bottles were placed in a slanting position till the agar solidified.

Identification and characterization of microorganisms

a. Grams staining

Gram staining was done to observe the shape of the cell and the type of cell wall it has. A loopful of water was placed in a grease free sterile slide and then a portion of the organism was spread to make a smear. The smear was air dried and heat fixed.

The smear was covered with crystal violet and allowed to stand for one minute, the stain was washed off and excess water was drained. The smear was covered with Grams iodine and allowed to stand for one minute. The excess iodine was drained off and rinsed gently. 75% alcohol was also used as a decolourizer and spread on the smear until the drops coming off the slide were a pale violet colour, for 20 seconds. The slide was washed gently with water. The smear was counterstained with safranin for 45 seconds. It was washed with water and the smear was allowed to blot dry. A drop of the immersion oil was placed on the smear and the slide was viewed under the microscope at the oil immersion objective. Gram positive cells appeared purple under the microscope and Gram negative cells appeared pink or red under the microscope.

b. Catalase test

The test organisms were streaked on Nutrient agar slopes and incubated for 3 days at 37^{o}C. A loopful of the culture was emulsified with a loopful of hydrogen peroxide (3%) on a slide. The formation of oxygen bubbles indicates the presence of catalase which means its catalase positive, and the absence of bubbles indicates a negative result, thereby making it catalase negative.

c. Motility test

This test is used in detecting motile and non-motile organisms. Under sterile conditions, nutrient agar was prepared at half strength in test tubes and sterilized. After the agar solidified, the test tube was stabbed with the test bacteria and incubated at 37^{o}C for 48 hours. Motile organisms moved away from the line of inoculation while non-motile organism grew along the stabbed lie with the medium remaining clear (21).

d. Citrate test

The citrate test detects the ability of an organism to use citrate as the sole source of carbon and energy.Under sterile conditions, Simmons Citrate Agar was inoculated lightly by using sterile inoculating pin from an 18-to-24-hour old colony. It was incubated at 35^{o}C for 3days. If a growth is observed on the medium, even without colour change, it will be considered as a positive growth. A colour change in the medium would be observed if the test organism produces acid or alkali during its growth. The usual colour change observed is from green (neutral) to blue (alkaline). No colour change (i.e. green) indicates a negative result.

e. Indole test

The test organisms were inoculated in the bottle containing peptone water and incubated for 5-7 days at 35^{o}C. After incubation, 2ml of chloroform was added to the broth and gently shaken. 2ml of Kovac's reagent was also added and mixed. A red colour in the alcohol layer indicates a positive reaction while a yellowing indicates a negative test (21).

f. Methyl red test

This test was used to detect if sufficient acid was produced by fermentation of glucose so that the pH of the medium falls and is maintained below 4.5.

The test organism was inoculated into sterile glucose phosphate broth (5ml) and incubated at 37^{o}C for 3 days. 5 drops of methyl red indicator solution was added after incubation, shaken and read immediately. A change from yellow to red indicated a positive result while a yellow colour indicates a positive result while a yellow colour indicates a negative result (21).

g. Vogesproskauer test

The isolate was grown in glucose phosphate peptone water as described for methyl red test. After incubation, 5mls of o'meara reagent i.e 40g KOH and 0.3g creatinine dissolved in 100 ml of distilled water

was added. The tubes were transferred to a 37C water bath for 4 hours, mixed and read within 30 minutes. The tubes were aerated by shaking at intervals. The development of an eosin-pink coloration indicated a positive vogesproskauer test.

h. Fermentation of sugar

4g of peptone water was dissolved in 100ml of distilled water, 1g of fermentable sugar was also dissolved and then 0.01 ml of phenol red (indicator) was added and then mixed properly. Six (6) ml of the medium was dispensed into test tubes, inverted durham tubes were placed in the tubes which was then autoclaved at 121^{o}C for 15 minutes.

Each test tube containing the different type of sugar was inoculated with a loopful of the different organisms and some of the tubes were left as control. The experimental and control tubes were incubated at37^{o}C for 5 days. The tubes were observed daily for acid production and for change in the colour of the indicator from red to yellow.

Antibiotic Susceptibility testing: The disc diffusion technique was used for antibiotic sensitivity testing using Mueller Hinton Agar. It was prepared according to the manufacturer's instruction. The agar was sterilized in the autoclave and allowed to cool before it was poured into sterile Petridishes and allowed to solidify using swab sticks, pure isolates were streaked uniformly on the agar slants and paper discs were impregnated with the antibiotic were placed on the surface of an agar plate previously inoculated with the test organism. The plates were incubated at 37^{o}C for 24 hours. After incubation, the organisms were checked for zones of inhibition and were recorded in millimetres.A clear zone of inhibition appeared where no growth was inhibited while in cases where no clear zones were seen, no inhibition occurred.

Results

The bacterial load of the water samples from Aiba andOke-Odo stream was studied.

Table 1 shows some of the features of the sample such as pH, conductivity and turbidity. The pH values for sample A ranged from 5.49-7.5, with a mean of 6.36, while pH values of sample B ranged from 5.54-6.9, with a mean of 6.36. This value did not affect the number of bacteria in the sample.

The conductivity values for sample A ranged from 31.1-437, with a mean of 180.78. The conductivity values for sample B ranged from 31.8-493, with a mean of 189.85. The conductivity values of the samples (A and B) from the first sampling were very low.

At 600nm, the turbidity transmission for sample A was between 78.3 and 103.9, with a mean of 91, while the absorbence ranged between 0.019 and 0.106, with a mean of 0.0405. At 600nm, the turbidity transmission for sample B was between 67.1 and 94.7, with a mean of 83.78, while the absorbence ranged between 0.025 and 0.173, with a mean of 0.081.

Table 1: Physicochemical analysis of Sample A (Aiba) and Sample B (Oke-odo).

Sample Code	Sampling	pH	Conductivity (uS/cm)	Turbidity	
				Transmission (600nm)	Absorbance (600nm)
A	1st	5.49	31.1	85.8	0.019
	2nd	6.23	437	96.0	0.018
	3rd	6.20	151.0	78.3	0.106
	4th	7.5	104.1	103.9	0.019
Mean		6.36	180.78	91	0.0405
B	1st	5.54	31.8	93.1	0.032
	2nd	6.30	493	67.1	0.173
	3rd	6.70	137.1	94.7	0.025
	4th	6.9	97.5	80.2	0.093
Mean		6.36	189.85	83.78	0.081

Table 2 shows the results of Biochemical Oxygen Demand (BOD) measurements of the samples. It was observed that BOD measurement for sample A ranged from 21.9-30.8It showed that the stream was a little bit contaminated. It was also observed that BOD measurement for sample B ranged from 72.5-91.5.It showed that the stream was very contaminated. This means that the microbes degrading organic matter were much.

Table 2: Biochemical Oxygen Demand (BOD) of Sample A (Aiba) and Sample B (Oke-odo).

Sampling	Sample Code	BOD(mg/l)
1st	A1	21.9
	B1	72.5
2nd	A2	25.4
	B2	89.9
3rd	A3	26.6
	B3	63.5
4th	A4	30.8
	B4	91.5

Table 3 shows the Gram staining reaction of the isolates, morphological and biochemical test of the isolates. The sugar fermentation profile of the isolates showed that all organisms fermented lactose with the production of gas. *Enterobacter* sp., *Escherichia* sp., and *Bacillus* sp. fermented all the sugars with the production of gas.

Table 3: Morphological and biochemical identification of isolated bacteria.

ISOLATES	GRAM STAIN	CATALASE	MOTILITY	CITRATE	INDOLE	MR	VP	LACTOSE	GALACTOSE	MANNITOL	GLUCOSE	PROBABLE ORGANISM
A1	+C	+	+	+	-	+	+	+A	+A,+G	+A,+G	+A,+G	*Staphylococcus*
A2	-C	+	+	-	+	+	+	-A	-A	+A,+G	+A,+G	*Serratia*
A3	-R	+	+	-	-	+	-	-A	+A,+G	+A,+G	+A,+G	*Salmonella*
A4	-R	-	-	+	-	+	-	+A,+G	+A,+G	+A,+G	-A	*Citrobacter*
A5	-C	+	+	+	-	+	+	+A,+G	+A,+G	+A,+G	+A,+G	*Enterobacter*
A6	-R	+	+	-	+	+	-	+A,+G	+A,+G	+A,+G	+A,+G	*Escherichia*
B1	-R	-	+	+	-	+	+	+A,+G	-A	+A,+G	-A	*Serratia*
B2	-R	+	+	+	-	+	-	+A,+G	+A,+G	+A,+G	-A	*Citrobacter*
B3	-C	+	-	+	-	+	-	+A,+G	+A,+G	+A,+G	+A,+G	*Citrobacter*
B4	-R	+	+	-	+	+	-	+A,+G	+A,+G	+A,+G	+A,+G	*Escherichia*
B5	-C	+	-	+	-	+	-	+A,+G	+A,+G	+A,+G	+A,+G	*Citrobacter*
B6	-C	+	-	+	-	-	+	+A,-G	+A,+G	+A,-G	+A	*Klebsiella*
B7	-R	+	-	+	-	+	-	+A,-G	+A,-G	-A	+A,+G	*Proteus*
B8	-R	+	+	-	+	+	-	+A,+G	+A,+G	+A,+G	+A,+G	*Escherichia*

KEY:

+C= Gram-positive cocci
-C= Gram-negative cocci
+A= Acid produced
+A,-G= Acid produced, No gas.
-R= Gram-negative rods
-A= No acid produced
+A,+G= Acid produced, Gas produced

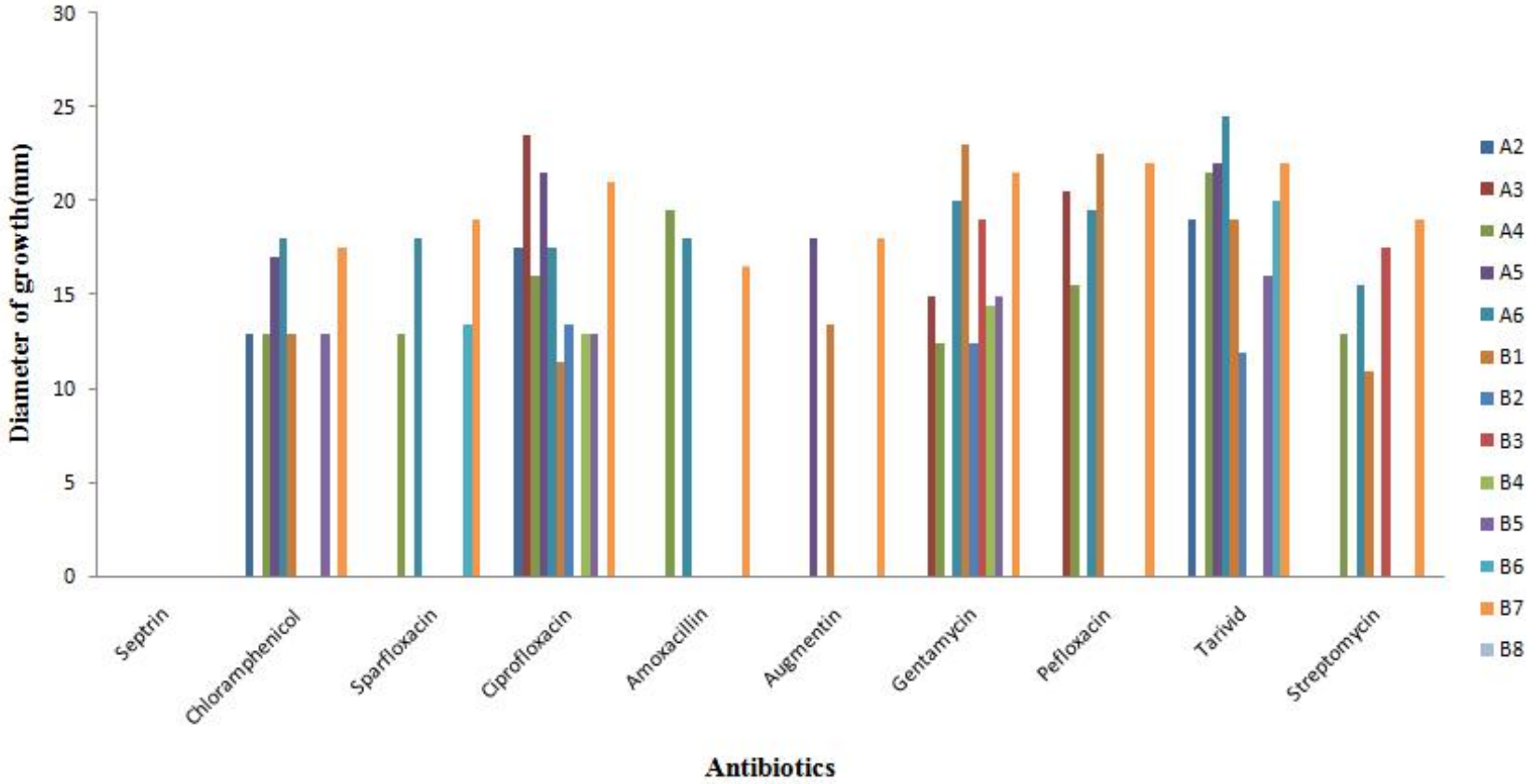

Figure 1: Antimicrobial susceptibility pattern of isolated Gram negative organisms using a Gram negative disc

Figure 1 shows the percentage resistance of Gram negative isolates to antibiotics. Isolate B8 was resistant to all the antibiotics. Isolate B3 was resistant to all the antibiotics, except gentamycin and streptomycin. Almost all the isolates were resistant to gentamycin and ofloxacin.All the isolates were resistant to septrin. Isolate B7 was sensitive to all the antibiotics, except septrin. Isolate A4 was sensitive to all the antibiotics, except septrin and augmentin.

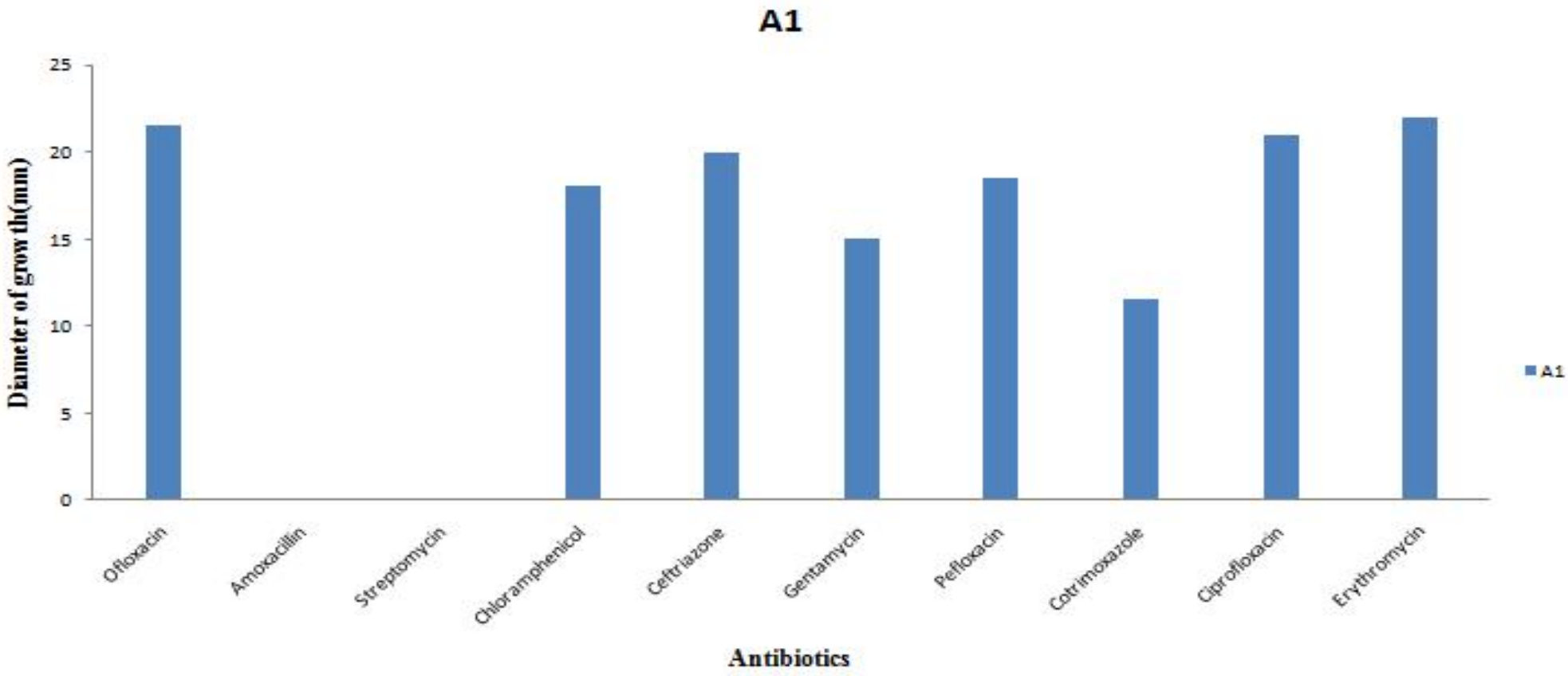

Figure 2: Antimicrobial susceptibility pattern of an isolated Gram positive organism using a Gram positive disc.

Plate1 shows Antibiotic sensitivity and resistance of Gram negative organisms to antibiotics.

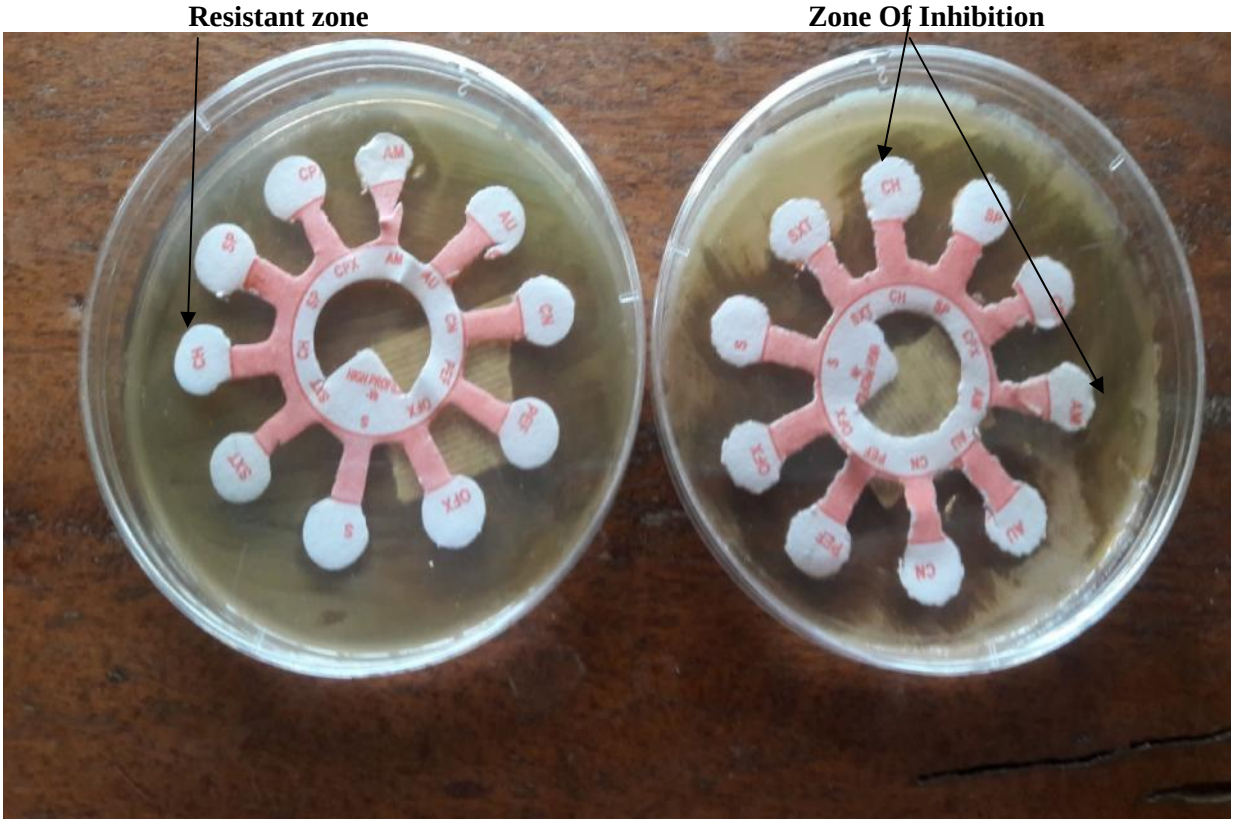

Plate 2 shows Antibiotic sensitivity and resistance of Gram positive organisms to antibiotics.

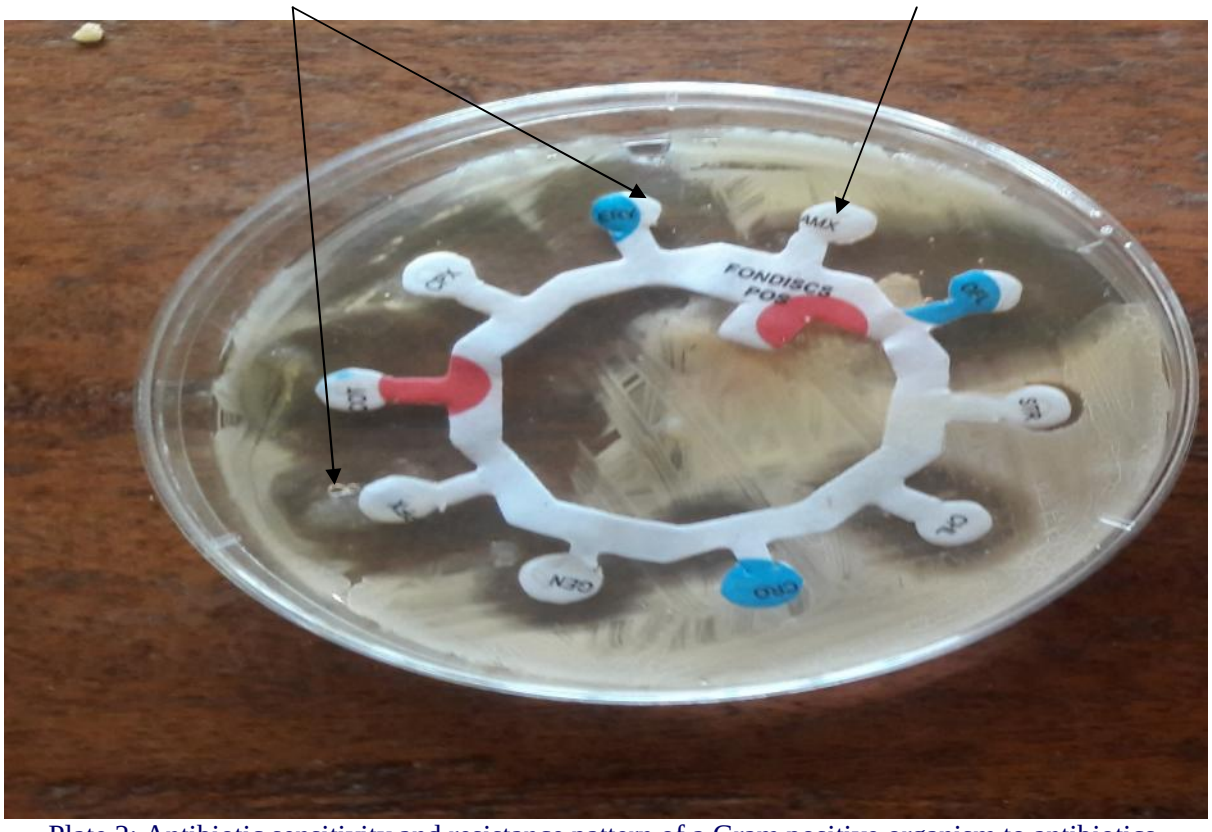

Plate 2: Antibiotic sensitivity and resistance pattern of a Gram positive organism to antibiotics.

Discussion

In developing countries, it is very difficult to get a water body that's free of contaminants. The bacterial contamination level is high in all the water samples. This result totally correlates with that of (14). The pH values in the water samples ranged from 5.49- 6.9. These values did not affect the number of the bacteria in the sample. However, it has been reported that pH values between 4 and 9 cause increase in faecal contamination (15). The Aiba River and the Oke-odo stream are sources of water for the citizens of Iwo. The isolates obtained from the water were found to be *Escherichia* sp., *Salmonella* sp., *Enterobacter* sp., *Citrobacter*sp., *Klebsiella* sp., *Proteus* sp., *Staphylococcus* sp.,and *Serratia*sp. Coliforms which typically reside in the intestine of both human and animals are used by regulatory agencies as indicator organisms of faecal pollution and their presence indicates a potential health risk because faecal-borne pathogens might also be present (16). The presence of pathogenic microorganisms most especially *Escherichia coli, Staphylococcus aureus,* e.t.c are attributed to poor hygiene and sanitary practices around the sources of water and the unavailability of facilities as well as financial constraints in the provision of water of good quality in rural areas (17).

In humans, *Klebsiella* sp. is present as commensal in the nasopharynx and in the intestinal tract. *Klebsiella* sp. can cause human diseases, ranging from asymptomatic colonization of the intestinal, urinary, or respiratory tract to faecal septicaemia. *Klebsiella. Pneumonia* and *Enterobacter aerogenes*(*K. Mobilis)* are mostly involved in nosocomial infections. In hospitals, the vectors are the hands of personnel and the reservoir is the gastrointestinal tract of patients (18). *Enterobacter cloacae* occur in the intestinal tract of humans and animals, in hospital environments, soil, water, sewage, e.t.c.

*Citrobacterfreundii*is an intestinal inhabitant of humans that may sometimes have or acquire the ability to produce an enterotoxin and thus become an intestinal pathogen. *Citrobacter*has been reported to be present in soil, sewage, water, food, e.t.c.

There have been many surveys of the occurrence on antibiotic resistant *Escherichia coli* in animals (19). Multiple drug resistance is an extremely serious health problemand it's associated with the outbreak of major epidemics around the world. The relatively high level of resistance to antimicrobial agents is a reflection of the misuse or abuse of these agents in the environment (20). Broad-spectrum antibiotics are sometimes given in place of narrow-spectrum drugs. All the organisms were resistant to septrin. Most of the organisms were resistant to amoxacillin, augmentin and sparfloxacin.

Salmonella, Enterobacter and Klebsiella were 100% resistant to septrin, amixacillin, and streptomycin. *Klebsiella* and *Samonella*were resistant to chroramphenicol. *Enterobacter and Klebsiella* 100% resistant to gentamycin. *Serratia*was 100% resistant to septrin, sparfloxacin and amoxacillin. It was 50% resistant to augmentin, gentamycin, perfloxacin and streptomycin. *E. coli* was mostly 66.66% resistant to all the antibiotics, except septrin. *Citrobacter*was 100% resistant to augmentin and septrin. *Staphylococcus* was resistant to amoxacillin and streptomycin.
This study has shown a steady and generalized increase in resistant bacteria to virtually commonly used antibiotics (antimicrobial agents).

Conclusion

Water is a valuable resource for plant and animals and it plays a vital role in their everyday activities. Intermittent water supply and sewage flood seem to contribute largely to self-reported diseases. Microbiologists are required to carry out the necessary tests and treatments to enable the availability of clean pure water in the Country. There should be further research on how to produce new antibiotics that pathogenic organisms are not resistant to. There should also be improvement on the old antibiotics, because without these, there would be great consequences on the world's public health. There should also be programmes to enlighten people on the use and misuse of antibiotics.

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