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Research Paper

Antibiotic Resistance *Escherichia* Strains Isolated From Well Water In Iree, Boripe Local Government, Osun State.

[1]Akinro, E. B., [1]Oladipupo, O. A.
[1]Abejoye, O. A., [2]Olakunle, T.P. and [3]Okemakin, F. Y.

[1]*Science Laboratory Technology Department*
[2]*Applied Sciences Department*
[3]*Biology Department, The Polytechnic, Ibadan. Oyo – State.*

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ABSTRACT:- This study was carried out to determine the incidence of antibiotic - resistant faecal indicator bacterial in ringed wells and unringed wells in Iree, Boripe Local Government Area of Osun State in South West Nigeria. Water samples from five ringed wells and five unringed wells at different parts of the town were collected over a month period. The total bacteria counts were performed using Most Probable Number (MPN) procedures and sensitivity of the isolates to antibiotics were tested. During the analysis, it was observed that both the ringed and unringed wells were badly contaminated based on the results of this work. This may pose a threat to human health due to the danger of water epidemic borne diseases and potential for the transfer of antibiotic resistance genes to pathogens and hence effective public health education aimed at creating awareness of the implication of consumption of contaminated and untreated water is imperative.

Keywords:- Antibiotic resistance, *E. coli* Strains, well water.

I. INTRODUCTION

Water is essential for man and other life forms. It is required for various human daily activities such as drinking, cooking, washing and also for agricultural and industrial purposes (1),(2). However, poor water quality continues to be a leading cause of health problems; especially in developing countries where it is estimated that 80% of all illness are linked to water and sanitation and 15% of all child deaths under the age of 5 years results from diarrhea diseases (2).

In Nigeria, increasing population and infrastructural breakdown have made municipal pipe borne water to be inadequate in quality and quantity (3). Today, less than 30% Nigerians have access to safe drinking water due to these inadequacies and most of the populations have to resort to drinking water from wells to streams especially all the rural and sub-urban communities (4),(5).

These diseases are caused by pathogenic bacteria, Viruses, Protozoa and other microbes which are shed in human faeces and pollute water supplies which people utilizes for drinking and washing purposes. Many rivers, streams and wells worldwide are affected by faecal contamination leading to increased health risks to persons exposed to the water, degradation of recreational and drinking water quality (6).

Pathogenic bacteria that may be associated with faecal contamination include *Escherichia coli; Campylobacter species, Salmonella species, Shigella species* and *Vibro cholera.* In addition to these organisms causing human diseases, resistance to antibiotics has made treatment to the disease, they cause more difficult (7),(8). Antibiotic resistance mechanism utilized by bacteria include the production of enzymes that degrade the antibiotics, even some bacteria can rapidly pump the antibiotics out of the cell before it has chance to interact within the cell. Also, some bacteria produce enzymes that inactivate the antibiotics by adding chemical structures on to the antibiotics (9),

Escherichia toll strains are members of the human and animal gut's natural flora. They are often isolated from water resources (swimming pools, water supply networks, lakes, sea water) contaminated

sewage outlets. The presence of E.coli.in water can be used as a microbiological indicator for faecal contamination and as a measurement or sanitary quality (10).

The overuse of antibiotics in human medicine and agriculture is a growing concern for public health overuse combined with inadequate waste water treatment has led to the presence of antibiotic resistance bacteria and genes encoding antibiotic resistance in surface waters, rivers sediment and the faeces of wild animals exposed to water residuals (11). The presence of antibiotic resistance bacteria in water resources throughout the world has been documented (12).

Aims and Objectives

The aims and objectives of the present work is to determine the incidence of antibiotics-resistant faecal indicator bacteria on ringed and unringed wells in Iree, Boripe Local Government Area of Osun State, South West, Nigeria.

II. MATERIALS AND METHODS

Water samples were collected from ringed wells and unringed wells. Five different samples for ringed wells and another five different samples for unringed wells. It was aseptically collected on steriles sampling bottles and immediately taken to Microbiology Laboratory of Osun State polytechnic free of analysis. The collection was made for over a month's period at 2ldays intervals.

Multiple tube test method was used for the analysis in which it took three stages, these are the presumptive test, confirmatory test and completed test. The presumptive test was prepared at single strength with sixty test tube filled with 10ml of nutrient broth each and then inoculated in ratio 1:1 with 0.1ml and 1ml of water sample respectively**.** Double strength was also prepared where thirty test tubes were used, filled with 10ml of nutrient broth and I Oral of water samples inoculated. Durham tubes were placed in inverted position to rudicate the production of gases provided coliforms are present. All samples are indicated for 28 - 28 hrs at 37^0C.

For the confirmatory test, a loopful of water sample in the positive presumptive test tube was streaked in Eosin Methylene Blue Agar and incubated at 37^0C for 24hrs. Growth of colonies will indicate positive confirmation and the appearance of greenish metallic sheen on the EMB agar plates will indicate the colonies of *E. coli*.

The completed test is done through nutrient agar where the colonies are screen again. The colonies on the EMB agar plates were streaked carefully on to the nutrient agar plates prepared. The plates were incubated at 37^0C for 24hrs. Also another hopeful from colonies from EMB afar on the confirmed test was inoculated into a single strength nutrient broth and incubated for 24-48 ohms at 37^0C.

Gram staining, catalase and coagulase test were carried out on the isolated strains of *E. coli*. Also, antibiotic susceptibility test was conducted with disc diffusion method. The number of coliforms is determined through MPN chart.

III. RESULTS AND DISCUSSION

RESULTS

Table 1: Obtainable results from the well water samples in Iree (first collection). 7th October 2013.

Sample	A	B	C	D
U1	10ml,1ml, 0.1ml	3-3-3 DS-DS-SS	0-1-0	3
U2	10ml, 1ml,0.1ml	3-3-3 DS-DS-SS	0-3-0	9
U3	10ml, 1ml, 0.1ml	3-3-3 DS-DS-SS	1-1-1	12
U4	10ml, 1ml, 0.1ml	3-3-3 DS-DS-SS	1-2-1	15
U5	10ml, 1ml, 0.1ml	3-3-3 DS-DS-SS	3-2-2	210
R1	10ml, 1ml, 0.1ml	3-3-3 DS-DS-SS	1-2-2	20
R2	10ml, 1ml, 0.1ml	3-3-3 DS-DS-SS	1-3-3	29
R3	10ml, 1ml,0.1ml	3-3-3 DS-DS-SS	2-1-3	34
R4	10ml, 1ml,0.1ml	3-3-3 DS-DS-SS	2-3-2	44
R5	10ml, 1ml,0.1ml	3-3-3 DS-DS-SS	3-2-2	160

Table 2: Obtainable result from well water samples in Iree (second collection). 28th October 2013

Sample	A	B	C	D
U1	10ml, 1ml, 0.1ml	3-3-3	0-2-0	6
		DS-DS-SS		
U2	10ml, 1ml, 0.1ml	3-3-3	0-2-2	7
		DS-DS-SS		
U3	10ml, 1ml, 0.1ml	3-3-3	1-0-2	11
		DS-DS-SS		
U4	10ml, 1ml, 0.1ml	3-3-3	1-0-2	19
		DS-DS-SS		
U5	10ml, 1ml, 0.1ml	3-3-3	0-3-3	24
		DS-DS-SS		
R1	10ml, 1ml, 0.1ml	3-3-3	1-0-0	4
		DS-DS-SS		
R2	10ml, 1ml, 0.1ml	3-3-3	1-1-0	7
		DS-DS-SS		
R3	10ml, 1ml, 0.1ml	3-3-3	1-0-1	9
		DS-DS-SS		
R4	10ml, 1ml, 0.1ml	3-3-3	1-0-1	0
		DS-DS-SS		
R5	10ml, 1ml, 0.1ml	3-3-3	2-1-0	15
		DS-DS-SS		

KEY: Sample Ul-U5 represent unringed well in five different locations
Sample RI-R5 represents ringed wells in five different locations
A – Amount of water sample inoculated.
B – Number of sample tubes of each test.
C – Number of tubes the five possible reaction.
D – Most possible number (MPN) determined.
Ds – Double strength of the medium
Ss – Single strength of the medium.

Table 3: Antibiotic sensitivity pattern of the strain (first collection) 7th October,2013

Antibiotic and code	Samples					
	U3 0.lml	U3 lml	U3 10ml	R3 0.1ml	R3 lml	R3 10ml
Septin sxt-30g	S	R	-	R	S	
Chloraphenicol CH-30g	R	R	-	R	R	R
Sparfloxacin Sp-10g	-		-	-	-	-
Ciprofloxacin Cpx-10g	R	S	-	R	R	R
Amoxocillin Am-30g	R	R	R	S	R	R
Augumentin Aug-30g	R		R	-	R	-
Gentamycin CN-10g	R	R	-	R	-	-
Pefloxacin PEF-30g	-	R	R	-	I	-
Tarivid OFX-10g	R	R	R	R	-	R
Streptomycin S-30g	R	-	R	-	R	-

Table 4: Antibiotic sensitivity pattern of the strains (Second collection). 21st October 2013.

Antibiotic and code	Samples					
	U3 0.1ml	U3 lml	U3 10ml	R3 0.lml	R3 1ml	R3 10ml
Septin sxt-30g	-	-	-	R	R	-
Chloraphenicol CH-30g	R	-	R	-	R	R
Sparfloxacin Sp-10g	-	-	-	-	-	-
Ciprofloxacin Cpx-10g	-	-	-	-	R	R
Amoxocillin Am-30g	R	-	R	-	-	R
Augumentin Aug 30g	-	-	-	-	-	R
Gentamycin CN-10g	-	-	-	-	-	-
Pefloxacin PEF-30g	R	R	R	R	R	R
Tarivid OFX-10g	R	R	R	R	R	R
Streptomycin S-30g	R	-	-	R	S	-

KEYS
R ----- Resistance
I ----- Intermediate
S ---- Susceptible
No reaction.

IV. DISCUSSION

The analysis of different five ringed wells and five unringed wells show the spread of diseases through faecal contaminants of the water sources. During the analysis, the MPN technique shows production of gases and acids which is the indication of coliform organism. This is reported by (13) that drawing water from wells could be a potential source of contamination as it may have had contact with human faecal matter.

Majority of the well water studied were without protective covers and various drawers were used in fetching the water which also resulted to high level of contamination. In this situation, the amount of chlorine to be used in disinfecting the water would be based with the level of coliform counts in the wells sampled, this is also reported by (14) that the level of contaminant in any water source would determine the amount of chlorine to be used for treating the water.

Furthermore, the gross contamination of well waters by pathogen might be due to openness and shallowness of the wells which allows easy entrance of particles from the surroundings, a practice that is common among the users since individuals bring along their own containers, and even, in study areas, public water supply is inadequate and irregular this is also reported by (14) that where there is inadequate supply of portable water, it leads to various activities by individuals concerning their respective homes.

The result of the antibiotic sensitivity test was measured around the zone of inhibition and strain was measured by 10mm wide around the disc which indicates that the antibiotic resistant is incomplete if the zone of inhibition is 16mm and it is regarded sensitive if it is 17mm narrow if the disc. Most of the antibiotics used by the *E. coli* strains.

Finally, antibiotic resistance in bacterial is a serious problem facing our society today and the reason for this is the overuse of antibiotics by human as reported by (13)

V. CONCLUSION

There is need to control faecal pollution of water supply to avert the occurrence of disease outbreak through effective public health education by relevant government agencies. People should be educated on the applications associated with the consumption of contaminated water for drinking and other domestic purposes.

VI. RECOMMENDATION

It is recommended that after drawn the water from the well, it must be properly boiled before use. Also, non- governmental agencies should channel efforts toward improving or providing safe drinking water supplies to the areas. The indiscriminate use of antibiotic an therapy should be avoided to parent the development of more antibiotic resistant bacteria strains. The wells should be chlorinated periodically in order to kill the terms.

There is need to control faecal pollution of water supply to avert the occurrence of diseases outbreak. Finally, antibiotic resistance infected people should avoid self medication, but seek proper

medical attention so that appropriate antibiotic can be administered rather than using drugs indiscriminately which can lead to resistance by organisms.

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