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RESEARCH ARTICLE

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MULTIPLE ANTIBIOTIC RESISTANCE INDEX OF ESCHERICHIA COLI ISOLATED FROM DRINKING WATER SOURCES IN ADO-EKITI, NIGERIA

ABSTRACT

Waterborne diseases are among the leading cause of morbidity and mortality in developing countries and could result from feacal contamination of surface water serving as source of drinking water in rural communities. The presence of *Escherichia coli* in water is an indication of faecal contamination. Hence, investigation on the incidence of antibiotic-resistant *E. coli* of different drinking water sources in Ado-Ekiti, Nigeria was carried out from June to November, 2011. Water samples were collected from different water sources (surface drinking water, water dams, shallow wells and commercial processed water) in Ado-Ekiti metropolis and analysed for microbiological quality. *Escherichia coli* strains were isolated from the water samples on Eosin methylene blue (EMB-agar). Two hundred and twenty eighty (228) strains were isolated from all the water sources and screened against eight antibiotics namely: amoxicillin, augmentin,gentamicin, cotrimoxazole, nitrofurantoin, nalixidic acid, ofloxacin and tetracycline. The mean antibiotic resistance patterns exhibited by the isolates were augmentin (91.7%), tetracycline (73.0%), nitrofurantoin (62.2%), cotrimoxazole (61.1%), gentamicin (45.0%) , nalixidic acid (38.7%), and ofloxacin (9.2%). Eleven (11) randomly selected antibiotic resistant *E.coli* strains that showed multiple resistance patterns were screened for plasmid, out of which ten (10) were found to harbour one or more plasmids of sizes 23.13kbp, 2.37kbp and 2.98kbp. High levels of MAR-index ranging from 0.81 to 3.08 as against low risk value of 0.2 could be an indication that all the water sources were of high public health risk in the study areas. Hence this study revealed the need for adequate water sanitation programme especially for rural communities to prevent outbreak of antibiotic-resistant waterborne infections.

Key words: Antibiotic resistance, Water sanitation, Public health risk, Multiple antibiotic resistance index.

INTRODUCTION

Contamination of surface water by antibiotic-resistant bacterial pathogens constitutes a serious environmental and public health threat (Torrella *et al.,* 2003). Waterborne infections are major global problem. It has been suggested that it`s the leading worldwide cause of deaths and diseases, accounting for the death of more than 14,000 people daily. Water pollution can be categorized into eight classes (National Water Bulletin, 1990). Some water pollution effects are recognized immediately, whereas others don't show up for months or years (Ashraf *et al.,* 2010). Estimation indicates that more than fifty countries of the world with an area of twenty million hectares area are treated with polluted or partially treated polluted water including parts of all continents and this poor quality water causes health hazard and death of human being, aquatic life and also disturbs the production of different crops. Infact, the effects of water pollution are said to be the leading cause of death for humans across the globe, moreover, water pollution affects our oceans, lakes, rivers, and drinking water, making it a widespread and global concern (Gauderman *et al.,* 2005).The microbiological safety of drinking water is of paramount importance to public health. Protection is generally acceptable as the primary strategy to obtain safe drinking water. However, many sources (surfaces water in particular) are highly contaminated and need extensive treatment before distribution to the consumers. The suitable indicators of faecal contamination of surface water are the coliforms, especially *E. coli* and *Streptococci*.

Water borne diseases are caused by pathogenic microorganisms which are directly transmitted when contaminated fresh water is consumed (Kahlown *et al*., 2004). Water borne diseases, such as cholera and typhoid fever, kill an estimated 5 million to 10 million people worldwide each year, according to the United Nations (Dziuban *et al*., 2006). Water borne infections are most common causes of morbidity and mortality in the underdeveloped and developing countries and 80% of the infectious diseases are water borne in most developing countries. It was

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estimated that 88% of global burden of disease is attributed to unsafe water supply, sanitation and hygiene. The use of antibiotics to combat those infections is a common practice, but indiscriminate use of antibiotics lead to antibiotic-resistance in microorganisms, which warrants the initiation of steps to prevent public health hazard (Tambekar and Banginwar, 2005). The most frequent occurring microbes in polluted water in most of the developed countries are *E. coli, Enterobacter aerogenes*, *Aeromonas* spp, and *Klebsiella* spp. (Okonko *et al.,* 2008). Most importantly, *E. coli* plays an important role in the sanitary analysis of water. *E.coli* is a gram negative, facultative anaerobic and non-sporulating organism. Most *E.coli* strains are harmless, but some, such as serotype O157: H7, can cause serious food poisoning in humans, and areoccasionally responsible for product recalls (Vogt and Dippold, 2005). Resistance genotypes so far characterized include genes for resistance to last-line antibiotics such as 3rd generation cephalosporins and fluoroquinolones (Alouache *et al.,* 2012; Wellington *et al.,* 2013; Tacão *et al.,* 2014).The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K2. *E. coli* are not always confined to the intestine, and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for faecal contamination (Thompson and Andrea, 2007).

Transmission of pathogenic *E. coli* often occurs via faecal-oral transmission. Common routers of transmission include: unhygienic food preparation, farm contamination due to manure fertilization, irrigation of crops with contaminated grey water or raw sewage (Heaton and Jones, 2008; Baig *et al.,* 2010), feral pigs on cropland (Thomas and Degregori, 2007), or direct consumption of sewage- contaminated water. Plasmid profile is necessary in order to identify the genetic material/components of each species of *E. coli* in water.
The objective of this study was to assess the health risk of surface water in Ado Ekiti metropolis in respect of incidence of antibiotic-resistant *E. coli*.

MATERIAL AND METHODS

Collection of samples

A total number of 93 samples of three (3) different water sources which included surface water, dams, shallow well, borehole water and commercial sachet water were collected from 31 different sites in Ado-Ekiti metropolis. The water samples were collected weekly over a period of 6 months from June to November, 2011. The water samples were transported on ice to the Microbiology Laboratory of Ekiti State University, Ado-Ekiti and analyzed within 6 hours of collection.

Determination of bacterial density in water samples:

The total bacterial density and total coliform counts were determine using pour plate method on Standard plate count agar and MacConkey agar as described by Fawole and Oso, 1996

Isolation and characterization of *E.coli*:

Isolation of *E. coli* strains was done on Eosin Methylene Blue agar and sorbitol MacConkey agar. Isolates were further characterized based on morphological and biochemical properties. Biochemical tests on the isolates were conducted as described by Olutiola *et al*. (1991).

Antibiotic sensitivity test

The Kirby-Baver method was used. In this method, each isolates of *E. coli* were standardized by introducing three pure colonies into 5ml of sterilized tryptone soy broth in different test tubes. The test tubes were incubated for 3-5 hours until the turbidity of the bacterial culture marched that of Barium sulphate (0.5 McForce turbidity.

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Mueller-Hinton agar was prepared according to the manufacturer`s instruction and sterilized at 121^{o}C for 15 minutes. This was cooled to 47^{o}C and poured aseptically into the appropriate petri-dishes. The petri-dishes were allowed to cool and the agar was allowed to set. The agar surfaces were then inoculated by swapping with test organism. The surface of the inoculated plates were allowed to dry and then placed aseptically with test antibiotic disc which were amoxycillin (AMX) -25µg, cotrimoxazole (COT) - 25µg, nitrofurantoin (NIT) -300µg, gentamicin (GEN) - 10µg, ofloxacin (OFL) - 30µg, augmentin (AUG) - 30µg, tetracycline (TET) - 30µg, and nalixidic acid (NAL) – 30µg . The plates were left for 30 min for the antibiotics to diffuse into the medium and were incubated at 37^{o}C for 24 hours. After which the diameters of the resulting zones of inhibition of growth were measured and recorded appropriately. The multiple antibiotic resistance (MAR) index was calculated as follows: MAR-index = a/bc where a is aggregate resistance score of all the isolates and b is the number of antibiotic tested and c is the number of strains screened for susceptibility to antibiotics. Value lower than 0.2 is is considered low risk while higher than 0.2 is indicated as high risk (Krumperman, 1983).

Plasmid analysis of selected isolates

Plasmid analysis some selected isolates of *E. coli* from all the water samples was conducted according to method described by Maniatis et al. (1989)

RESULTS AND DISCUSION

The total bacterial count of all the samples collected ranged from 3.39 to 6.27 $\log_{10}$ cfu/ml. The highest bacterial count 6.27 $\log_{10}$cfu/ml was found in water dam samples, while the lowest count was found in commercial processed water samples. The total coliform count of all the samples collected ranged from 2.30 to 6.21 $\log_{10}$cfu/ml. The highest coliform count was found in deep well (borehole) water samples and the lowest was found in commercial processed water samples. The TBC/TCC ratios were found to range from 0.08 to 0.94 **(**Table 1**).**

A total of 228 isolates of E. *coli* were obtained from surface water (63), water dam (36), shallow well (71). Borehole water (36) and commercial processed water (22). In general the mean antibiotic resistance patterns of *E. coli* strains all the water sources were augmentin (91.7%), tetracycline (73.0%), nitrofurantoin (62.2%), cotrimoxazole (61.1%), gentamicin (45.0%) , nalixidic acid (38.7%), and ofloxacin (9.2%). The MAR-index exhibited by the E.coli strains from the five water sources were 0.81, 2.10, 0.98, 1.14 and 3.08 respectively (Figure 1) and as such are evidence of public health risk. Ten out of 11 isolates were observed to harbor 2 to 3 plasmids with molecular sizess 23.13kbp, 2.37kbp and 2.98kbp Table 4 and plate 1).

Water-borne infections have remained a significant health hazard in the world causing a great deal of public health concern. These water borne infections and diseases are known to be caused by some organisms associated with a wide variety of waters. *E. coli* isolated from the water samples were found to show high level of resistance to most antibiotics used. Antibiotics resistance will not only complicate future antibiotics therapy, but can also potentially stimulate the transfer of resistance genes. The presence of *E. coli* in drinking water is an indication of faecal contamination and can represent a risk of water-borne diseases.

The presence of MAR *E.coli* strains in the water samples may be attributed to poor sanitary and poor hygienic conditions of the sampling sites, poor hygienic condition of the people living around the sampling sites, contamination by domestic animals and industrial activities around the sites, inadequate waste disposal facilities and refuse/sewage disposal around the sampling sites. It is noteworthy that MAR-index of all the isolates from from the five water sampling sources were extremely high which ranged from 0.81 to 3.08 thus indicating that the 5 water sources were of high risk to public health. This signaled the exposure of water source to high risk contamination with the increased possibility of transferring harmful microorganisms to human. The high MAR index value may be due to the widespread use of antibiotics. The continuous use of a single antibiotic over a period of weeks or months

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will select bacteria with resistance to different kind of antibiotics. Conjugation that may occur among species or between species will build resistance diversity towards antibiotics (Tamanai-Shacoon *et al.,* 1995).

However, several studies have reported the presence of intergrons and gene cassettes among clinical isolates of *E. coli*; development of different antibacterial mechanisms of action and acquisition of resistance through genetic recombination: conjugation, transduction, transformation and evolution (Miranda ***et al.,* 2004**).
Uncontrol use of most of the antibiotics as a result of their affordability and frequent availability, could have lead to increase resistance to nalixidic acid, gentamicin and cotrimoxazole and this could have been the case for decades (Fey *et al.,* 2000).

Plasmid profile analysis of *E. coli* isolates revealed that most of the isolated *E.coli* that were resistant to two or more antibiotics harbored one or more plasmids. This was similar to what was observed by Smith *et a.l* (2003) and Umolu *et al.* (2006). They isolated three (3) plasmids from cow and two 2 from beef. The three (3) plasmids isolated from cow were 23.13kbp, 4.361kbp and 0.564kbp, while the two (2) plasmids isolated from beef were 23.1kbp and 4.36kbp.

This study therefore reveals the urgent necessity for adequate monitoring of the presence of drug resistant pathogens in public water supply to control the spread of waterborne infections especially in rural community where access to good quality water are not regularly available.

Table 1:Bacterial population of different water samples sources in Ado-Ekiti.

Water sample sources (n)	Bacterial density of water samples (log10cfu/ml)		TCC/TBC Ratio
	TCC	TBC	
Surface water (10)	4.05	5.39	0.79
Water dams (5)	5.95	6.27	0.47
Shallow wells (10)	3.68	3.83	0.70
Boreholes (10)	6.21	6.14	0.94
Processed water (13)	2.30	3.39	0.08

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Table 2: Antibiotics resistance of *Escherichia coli* isolated from surface drinking water in Ado-Ekiti.

ANTIBIOTIC(µg/disc)	Antibiotic resistance patterns (%)					Mean Antibiotic resistance pattern (%)
	Surface water (n=63)	Water Dam (n=36)	Shallow well water (n=71)	Borehole water (n=36)	Commercial processed water (n=22)	
Amoxycillin	97	97.2	100.0	88.9	100.0	96.6
Cotrimoxazole	52.5	88.9	99.1	22.2	95.5	61.1
Nitrofurantoin	53	86.1	60.9	61.1	50.0	62.2
Gentamicin	34.9	75.0	53.7	25.0	36.4	45.0
Nalixidic Acid	30.6	55.5	37.9	19.4	50.0	38.7
Ofloxacin	5.5	16.7	9.6	0.0	14.0	9.2
Augmentin	83.5	97.2	100	77.8	100.0	91.7
Tetracycline	50.1	86.1	100.0	33.3	95.5	73.0

Key: n= Total number of *E.coli* isolated from collected water samples

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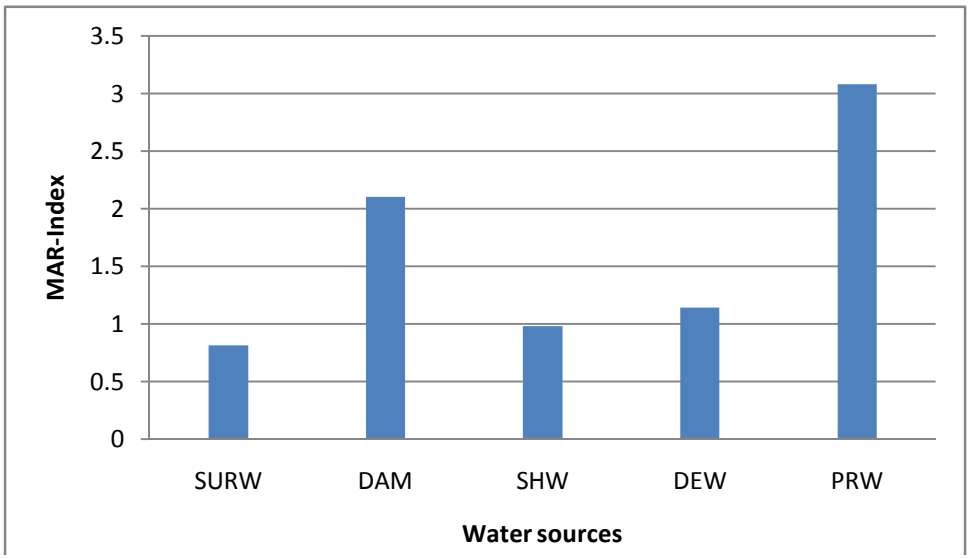

Figure 1: Multiple antibiotic resistance index of Escherichia coli from difference water sources (SURW, Surface water; DAM, Water Dam; SHW, Shallow well; DEW, Deep well and PRW, Process water)

Table 4: Multiple antibiotic resistance (MAR) patterns of *Escherichia coli* isolated from drinking water samples

Water samples sources	Multiple antibiotic resistance patterns (%)							
	**SR-1	MAR-2	MAR-3	MAR-4	MAR-5	MAR-6	MAR-7	MAR-8
Surface water (n = 63)	0.00	35.5	42.2	6.60	6.60	6.60	2.20	2.80
Dam water (n = 36)	2.80	0.00	2.80	2.80	19.4	38.9	25.0	0.00
Shallow well (n = 71)	0.00	0.00	3.60	35.7	44.6	10.7	1.80	0.00
Borehole water (n = 36)	2.70	11.1	58.3	13.8	0.00	0.00	0.00	0.00
Commercial processed water (n = 22)	0.00	4.50	0.00	13.6	36.4	27.3	9.10	0.00

**** Single-R type**

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Table 5: profiles and molecular size of plasmids selected *Escherichia coli* from water samples.

E. coli Strains	Identified Resistotypes	Number of plasmids	Observed Molecular sizes of plasmids
E1	*amx,/aug*	3	23.13, 2.37, 2.98
E9	*amx/nit/gen/nal/aug/tet*	2	23.13, 2.98
E15	*amx/cot/nit/nal/aug/tet*	1	23.13
E16	*amx/cot/nit/nal/aug*	3	23.13, 2.37, 2.98
E19	*amx/cot/nit/gen/nal/aug/tet*	3	23.13, 2.37, 2.98
E21	*amx/nit/gen/aug*	3	23.13, 2.98, 2.98
E23	*amx/nit*	3	23.13, 2.37, 2.98
E27	*amx/cot/nit*	2	23.13, 2.98
E38	*amx/nit/aug*	0	-
E40	*amx/nal*	3	23.13, 2.37, 2.98
E45	*amx/gen/aug/tet*	3	23.13, 2.37, 2.98

Key: kbp=Kilobase pair, Amx=Amoxycillin, Aug=Augmentin, Nit=Nitrofurantoin, Gen=Gentamicin, Nal=Nalixidic Acid, Tet=Tetracycline, Cot=Cotrimoxazole, E=*E.coli*

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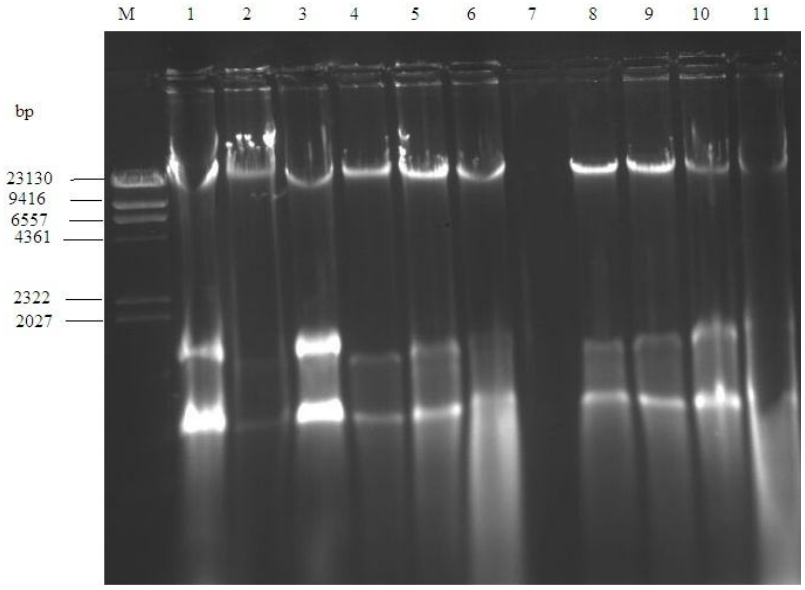
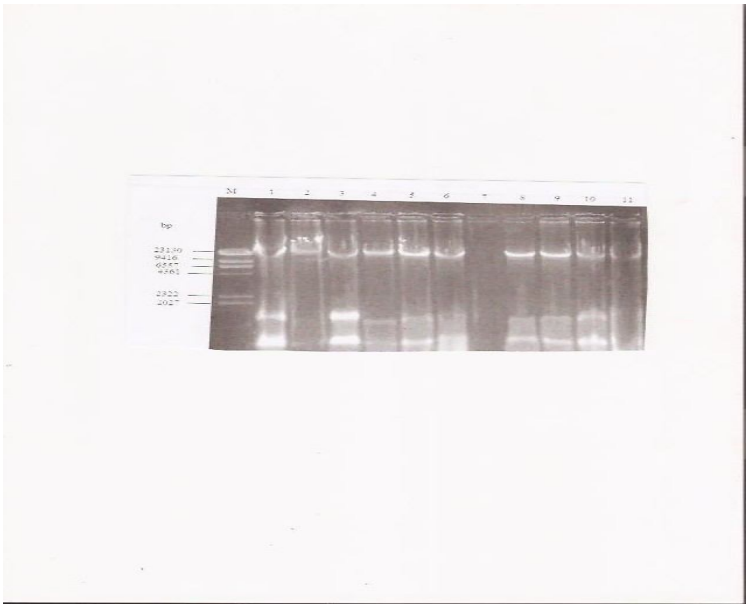


Key: M= HindIII Digest Marker, bp=base pair

Plate. 1: Plasmid profile of *Escherichia coli* isolated from different water samples in Ado-Ekiti.



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