

# AN ASSESSMENT OF MULTIDRUG RESISTANT BACTERIAL STATUS OF OGANE-AJI RIVER, ANYIGBA, KOGI STATE

## Abstract

Surface aquifers can be useful for a variety of purposes, but they can also act as fomites. River water could be a major route of spread of disease causing and multidrug resistant bacteria. The multidrug resistance pattern of bacteria from the Ogane-Aji River was studied. The river water samples were collected at two different points namely, Point A (10 cm depth) and Point B (45 cm depth). Standard microbiological techniques were used to identify the bacterial isolate that was obtained. The disks agar diffusion method was used to examine the isolates for resistance to nine foreign antibiotics and 20 indigenous antibiotics. These samples were analyzed by the most probable number (MPN) of which point B had more MPN index/ml (180) than point A which had 79 MPN/index/ml. The organisms most frequently isolated from the samples included those of the genera *Staphylococcus*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Escherichia*, *Klebsiella*, *Streptococcus* and *Salmonella*. Percentage resistance to all the foreign antibiotics by the isolates were obtained {*Staphylococcus* sp.(55.5%), *Bacillus* sp. (66.6%), *Enterobacter* sp.(77.8%), *Streptococcus* sp. (88.8%), *Pseudomonas* sp. (66.6%), *E. coli*, *Klebsiella* (77.8%), *Streptococcus* sp.(88.8%) and *Salmonella* sp. (88.8%) while percentage resistance to all the indigenous antibiotics were *Staphylococcus* sp.(30%), *Streptococcus* sp.(20%), *Bacillus* sp.(70%), *Enterobacter* sp. (60%), *Pseudomonas* (50%), *E. coli* (50%), *Klebsiella* (40%) and *Salmonella* (20%)}. It was discovered that every isolate was multidrug resistant. It is concerning for people's health that these organisms are present in this body of water, which is used for numerous things in the area. The river water could be a means whereby these etiologic agents and the multidrug resistance properties can spread through the population in contact with the river.

**Key words:** agar diffusion, antibiotics, bacteria, multidrug resistance.

## Introduction

“The emergence of multidrug-resistance bacteria limits the clinical use of antibiotics and, as resistant bacteria become more prevalent, there is increasing concern that existing antibiotics will become ineffective against these pathogens and more expensive. Multidrug-resistance genes conferring resistance to a wide variety of organisms have been identified in a large range of water environments including drinking water in both developed and developing countries” (Argerso, 2007). “The main risk for public health is that resistance genes are transferred from environmental bacteria to human pathogens. The potential of drinking water to transport microbial pathogens to a greater number of people, causing subsequent illness, is well documented in countries at all levels of economic development” (Argerso, 2007).

“According to the World Health Organization, 80% of all diseases are attributed to unsafe water. Developing countries in particular, are plagued with water-related diseases such as diarrhoea which account for 10% of the disease burden in such countries” (WHO, 2010).

“Antimicrobial resistance (AMR) presents a major and growing threat to effective treatment of bacterial infections. For almost a century antimicrobials have been used to control bacterial infections and disease in humans and animals. However, with increasing microbial resistance to drugs, we are gradually returning to nineteenth century levels of morbidity” (Hawkey, 2008; Chan, 2011). “Hitherto, investigation and policy development for the control of AMR using surveillance data have focused largely on patterns of resistance to individual antimicrobials” (Skjõt-Rasmussen *et al.*, 2009). “Quantitative studies have concentrated on theoretical frameworks using simulated and in vitro experimental data” (Smith *et al.*, 2002; Bergstrom *et al.*, 2004), “while much of our current understanding of the impacts of individual antimicrobials has been derived from small scale clinical epidemiological studies” (Jiang *et al.*, 2006).

Only recently, the broader ecological landscape occupied by the bacteria and their hosts has been considered by Singer *et al.* (2006) and Martinez (2008). “Novel approaches may bring

new perspectives on the origins and spread of AMR, or assist in the development of new or revised targeted interventions. The use of antimicrobials in agriculture as a major driver of AMR in pathogenic bacteria of significance to humans is an issue over which opinions are divided” (Angulo *et al.*, 2004; Wassenaar, 2005). “The prophylactic and metaphylactic use in animal populations has been a particular concern ,especially when the drug classes are the same as or related to the pharmaceuticals used in the control of human infections. Exposure of microbial populations to antimicrobials evidently selects for resistance; however, the critical and unresolved issue is **the** relative contribution to resistance in these populations from the different **sources**” (Aarestrup *et al.*, 2001).

The multidrug resistance mechanism employed by bacteria has resulted in a serious public health threat today. River water is the major source of water for household use in most rural communities in Kogi State. It is used for various purposes such as drinking, cooking, bathing and laundry; hence it serves as a perfect medium for spreading these bacteria. River contamination occurs when waste and different other pollutants are discharged into river without being properly treated (Abraham, 2010).

“Multidrug resistance is rising to dangerously high level in all parts of the world. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases. A growing list of infections – such as pneumonia, tuberculosis, blood poisoning, gonorrhoea and food borne diseases are becoming harder and sometimes impossible, to treat as antibiotics become less effective” (WHO, 2010).**There is need to monitor our aquifers for multidrug resistant bacteria and to create the much needed awareness in order to alleviate the spread of multidrug resistance.**

## **Materials and methods**

### **Study Area and Sample Collection**

The study area is Ogane-Aji, a village in Dekina Local Government, Kogi State. That is the source of Ogane-Aji River. The water sample (20) was randomly collected (50 ml) into a sterile glass bottle at two different points. Point A (10 cm depth) and Point B (45 cm depth) 50 metres apart along the course of the river. The depth is the area within which water is regularly taken for domestic uses. The bottled water were placed in ice-boxes and taken to the Microbiology Laboratory, Kogi State University, Anyigba for further analysis.

### **Dilution Preparation and Inoculation**

Serial dilution was carried out on the water samples by introducing 1 ml sample into 9 ml sterile distilled water. This was repeated with the dilution made to get various dilutions up to dilution 5. Appropriate dilution (1 ml) was used to inoculate cool, sterile, molten nutrient agar using the pour plate method and incubated at 37°C for 2 days. Total coli form count was estimated using the most probable number (MPN) method.

### **Bacterial Isolation and Identification**

Distinct colonies from the mixed culture plates were sub-cultured severally to get a pure bacterial isolate. The bacteria were identified using Gram test and biochemical tests (catalase, methyl red, indole, Voges-Proskauer, oxidase, citrate and urease) as described by Nester *et al.* (2007).

### Antibiotic Susceptibility Testing of the Isolates

Antibiotic susceptibility test was carried out using disk diffusion method on Mueller Hinton agar plates. The identification of Multidrug Resistance Isolates was recorded through the zone of inhibition measured to the nearest millimetres.

### Results

The Gram reaction and biochemical characteristics of the pure bacterial isolates from Ogane-Aji River water is shown in Table 1. A total of eight organisms were isolated. The isolates from the Ogane-Aji water sample showed that 75% are rod shaped and mostly enterobacteriaceae members as shown in Table 1. The organisms are predominantly Gram negative.

**Table 1:** Gram reaction and biochemical characterization of pure isolates

Isolates	Gram	Cell	Biochemical Tests							Probable organism
	Reaction	shape	CAT	INDO	CIT	MR	UR	OXI	VP	
A <sub>1</sub>	+	Cocci	+	-	+	+	-	-	+	<i>Staphylococcus</i> sp.
A <sub>2</sub>	-	Rod	+	-	+	+	-	-	-	<i>Bacillus</i> sp.
A <sub>3</sub>	-	Rod	+	+	+	-	-	-	+	<i>Enterobacter</i> sp.
A <sub>4</sub>	-	Rod	+	-	+	-	-	+	-	<i>Pseudomonas</i> sp
B <sub>1</sub>	-	Rod	+	+	-	+	-	-	-	<i>Escherichia coli</i>

B <sub>2</sub>	-	Rod	+	-	+	-	+	-	+	<i>Klebsiella</i> sp.
B <sub>3</sub>	+	Cocci	-	-	-	+	+	-	+	<i>Streptococcus</i> sp.
B <sub>4</sub>	-	Rod	+	+	-	-	-	-	-	<i>Salmonella</i> sp.

KEY: CAT = Catalase OXI = Oxidase INDO = Indole URE = Urease CIT = Citrate

MR = Methyl Red VP = Voges-Proskauer

Table 2 showed the results of the antibiotic susceptibility test carried out on the pure isolates from the Ogane-Aji River water using the imported antibiotic disks. *Staphylococcus* sp.

showed the least resistance of 55.5% while *Streptococcus* sp. and *Salmonella* sp. displayed

highest resistance of 88.8% to the tested antibiotics as showed in Table 2

**Table 2:** Antibiotic susceptibility test of isolates (imported disks)

Chemical class of antibiotics	Antibiotics	<i>Staphylococcus</i> sp.	<i>Bacillus</i> sp.	<i>Enterobacter</i> sp.	<i>Pseudomonas</i> sp.	<i>E. coli</i>	<i>Klebsiella</i> sp.	<i>Streptococcus</i> sp.	<i>Salmonella</i> sp.
β-lactam	AMP(10μg)	I	R	R	R	R	R	R	R
	OX(1μg)	R	R	R	R	R	R	R	R
	AUG(30μg)	R	R	R	R	S	R	R	S
Macrolide	VAN(30μg)	S	S	S	S	R	I	R	R
Cephalosporin 3 & 4 generation	CAZ(30μg)	R	R	R	R	R	R	R	R
	CTX(30μg)	R	R	R	R	R	R	R	R
Tetracycline	TE(30μg)	R	R	R	R	R	R	R	R
	DO(30μg)	S	S	R	R	R	R	R	R
Chloramphenicol	C(30μg)	S	S	I	S	S	I	S	R
	Susceptible	33.3%	33.3%	11.1%	22.2%	22.2%	0%	11.1%	11.1%
	Intermediate	11.1%	0%	11.1%	11.1%	0%	22.2%	0%	0%
	Resistant	55.5%	66.6%	77.8%	66.6%	77.8%	77.8%	88.8%	88.8%

KEY: AMP = ampicillin OX = oxacillin VAM = vancomycin AUG = amoxicillin/clavulanic acid C= chloramphenicol CAZ = ceftazidime CTX = cefotaxime TE = tetracycline DO = doxycycline S = susceptible I = intermediate R = resistant

Table 3 showed the antibiotic sensitivity of Gram positive isolates to the indigenous antibiotic disks. The range of resistance was between 20 – 30%.

**Table 3:** Antibiotic susceptibility of gram positive isolates (indigenous disk)

Chemical Class of Antibiotics	Antibiotics	<i>Staphylococcus sp.</i>	<i>Streptococcus sp.</i>
Fluoroquinolones	CPX(10µg)	S	S
	NB(10µg)	R	I
	LEV(20µg)	S	S
Aminoglycosides	GN(10µg)	S	S
	S(30µg)	I	S
Macrolide β-lactam	E(30µg)	S	S
	AML(20µg)	I	R
	APX(20µg)	I	R
Antitubercular Chloramphenicol	RD(20µg)	R	I
	CH(30µg)	R	I
	Susceptibility	40%	50%
	Intermediate	30%	30%
	Resistant	30%	20%

**KEY:** CPX = ciproflox NB = norfolaxin GN = gentamycin AML = amoxil S = streptomycin  
RD = rifampicin E = erythromycin CH = chloramhenicol APX = ampiclo, LEV = levofloxacin

The antibiotic sensitivity of the Gram negative isolates from Ogane-Aji River is shown in Table 4. Out of the Gram negative rods tested, *Klebsiella* and *Salmonella* had resistance of 40% and 20%, respectively while others showed resistance between 50% and 70%.

**Table 4:** Antibiotic susceptibility of Gram-negative isolates

Chemical Class of Antibiotics	Antibiotics	<i>Bacillus sp.</i>	<i>Enterobacter sp.</i>	<i>Pseudomonas sp</i>	<i>E. coli</i>	<i>Klebsiella sp</i>	<i>Salmonella sp</i>
Fluoroquinolones	OFX(10µg)	S	R	S	S	S	S
	CPX(10µg)	S	R	S	R	S	S
	NA(30µg)	R	I	R	R	R	I
β-lactam	AU(20µg)	R	I	S	R	I	S
	PN(20µg)	R	R	R	S	R	I
Aminoglycosides	GN(30µg)	R	S	R	R	R	R
	S(20µg)	R	R	R	S	I	I
Cephalosporin	CEP(30µg)	R	R	R	R	R	I
Sulfonamides	SXT(20µg)	S	R	S	I	I	S
Reflacine	PEF(10µg)	R	S	S	I	I	R
	Susceptibility	30%	20%	50%	30%	20%	40%
	Intermediate	0%	20%	0%	20%	40%	40%
	Resistant	70%	60%	50%	50%	40%	20%

**KEY:** OFX = tarivid PEF = reflacine CPX = ciprofloxacin AU = augmentin GN = gentamycin S = streptomycin. CEP = ceporex NA = nalidixic acid SXT = septrin PN = ampicilin

The Most Probable Number (MPN) results for the estimation of number of bacteria per 100 ml of the water sample is shown in Table 5.

**Table 5:** MPN of the bacteria from Ogane-Aji river water

Sample site	No. of tubes giving			MPN index per 100ml	95% confidence limit	
	10ml	1ml	0.1 ml		low	High
A	5	3	0	27	9	80
B	5	3	3	33	11	93

## Discussion

Water sample collected from Ogane-aji river in Anyigba Kogi State was used to assess the multidrug resistance status of the river. Enumeration and isolation of bacteria was done and a total of eight bacterial genera were isolated (Tables 3 and 4). Characterization and identification of bacteria isolates was done through Gram staining and biochemical test shows that two organisms were Gram positive (Tables 3) while six organisms were Gram negative (Tables 4).

The antibiotic susceptibility and resistance pattern (Table 2) for imported disk showed that all the isolates were resistant to not less than five out of the nine tested antibiotics, this is of grave health concern. Sensitivity of 33.3%, 11.1% intermediate resistance and 55.5% resistance were observed for *Staphylococcus* sp., *Bacillus* sp. showed 33.3% sensitivity, 0% intermediate and 66.6% resistance. *Enterobacter* showed 11.1% sensitivity, 11.1% intermediate and 77.7% resistance. *Pseudomonas* showed 22.2% sensitivity, 11.1% intermediate resistance and 66.6% resistance. *E. coli* also showed 22.2% sensitivity, 0% intermediate resistance and 77.7% resistance. *Klebsiella* showed 0% sensitivity, 22.2% intermediate resistance and 77.8% resistance. *Streptococcus* showed 11.1% sensitivity, 0% intermediate resistance and 88.8% resistance. *Salmonella* had 11.1% intermediate resistance

and 88.8% resistance, respectively. *Staphylococcus* showed resistance to oxacillin, amoxicillin, cefotaxime, ceftazidime and tetracycline. Intermediate resistance to ampicillin and susceptible to vancomycin, chloramphenicol and doxycycline.

*Bacillus* sp. showed resistance to ampicillin, oxacillin, amoxicillin, ceftazidime, cefotaxime and tetracycline. Susceptible to vancomycin, chloramphenicol and doxycycline. *Enterococcus* showed resistance to ampicillin, oxacillin, amoxicillin, ceftazidime, cefotaxime, tetracycline and doxycycline and intermediate resistance to only chloramphenicol while susceptible to only vancomycin. *Pseudomonas* showed resistance to ampicillin, oxacillin, amoxicillin, ceftazidime, cefotaxime, tetracycline and intermediate resistance to only doxycycline but showed susceptibility to vancomycin and chloramphenicol. *E. coli* showed resistance to ampicillin, oxacillin, vancomycin, ceftazidime, cefotaxime, tetracycline and doxycycline. Susceptibility to amoxicillin and chloramphenicol was also shown by *E. coli*. *Klebsiella* showed resistance to ampicillin, oxacillin, amoxicillin, ceftazidime, cefotaxime, tetracycline and doxycycline. It also displayed intermediate resistance to the macrolide and imported chloramphenicol tested. *Salmonella* also showed resistance to all the imported antibiotics used except amoxicillin which it was susceptible to.

The bacterial susceptibility and resistance pattern (Table 3 and 4) to commercially available indigenous antibiotic disks showed 50% susceptibility to ciprofloxacin, gentamycin, Erythromycin, streptomycin and Levofloxacin. Intermediate resistance of 30% to Amoxicillin, Streptomycin and Ampiclox. 20% resistance to Norfloxacin, and Rifampicin for *Staphylococcus* sp. *Streptococcus* sp. showed 50% susceptibility to ciprofloxacin, streptomycin, gentamycin, erythromycin and Levofloxacin. Intermediate resistance of 30% to norfloxacin, rifampicin and chloramphenicol. Resistance (20%) to amoxicillin and ampiclox. *Staphylococcus* sp showed 40% susceptibility to ciprofloxacin, gentamycin, erythromycin and Levofloxacin. The organism had 30% intermediate resistance to streptomycin, amoxicillin and ampiclox. It also had 30% resistance to norfloxacin, rifampicin and chloramphenicol. *Bacillus* sp showed 70% resistance to rifampicin, augmentin, gentamycin, streptomycin, cefepime, nalidixic acid and ampicillin, an intermediate resistance of 0% and 30% susceptibility to

tarivid, ciproflox and septrin. *Pseudomonas* sp showed 50% resistance to gentamycin, streptomycin, ceporex. nalidixic acid, ampicillin and intermediate resistance of 0% with 50% susceptibility to tarivid, ciproflox, augmentin, reflacine and septrin. *Kelbsiella* showed 40% interemediate resistance to reflacine, augmentin, streptomycin and septrin. It also had 20% susceptibility to tarivid and ciproflox. *Salmonella* showed 20% resistance to reflacine and gentamycin, 40% intermediate resistance to streptomycin, ceporex, nalidixic acid and Ampicillin. However it had 40% susceptibility to tarivid, ciproflox, augmentin and septrin.

The pattern of resistance in this study to several key antibiotics commonly used in therapeutic treatment is considered a public health threat. The isolation of *E. coli* multidrug resistance bacteria from Ogane-Aji River correlates with the work of Stephen and Kennedy (2018) who isolated multidrug resistance *E. coli* from water sources. Multidrug resistance patterns have been also detected in *E. coli* isolated from river water in Osun State Nigeria, (Titilayo *et al.*, 2015) and from Yamuna river in the holy city of Mathura, India (Amit *et al.*, 2014). The isolation of multidrug resistant *Enterobacter*, *Klebsiella*, and *Streptococcus* from Ogane-aji River correlates with the work of Abdul-Rasheed and his co-workers (2013) that isolated similar multidrug resistance organisms from Opa River, South-Western Nigeria.

The incidence of multidrug resistance isolates from the *Enterobacteriaceae* family from Ogane-aji river Anyigba correlates with the work of Graham *et al.*, (2014) who identified multidrug resistant isolates from the *Enterobacteriaceae* family in Almendares River in Cuba.

As shown in this study, the imported disks were found to be more potent than the indigenous disk. The organisms (Table 2) showed a higher resistance range of 55.5% - 88.8% to imported disk than the indigenous disk with 20 -70%. Higher level of intermediate resistance was also recorded for the indigenous antibiotic disks.

The presence of faecal coli forms in water samples serve as indicators of faecal contamination (Agunwamba 2000) and could be responsible for presence of other intestinal pathogens. *Streptococcus* sp, *Staphylococcus* sp, *Salmonella*, *Pseudomonas* and *Bacillus*

which were isolated and identified from the samples are pathogens of importance that have been linked to gastrointestinal disorders (Nwidu *et al.*, 2008)

The results of the present study demonstrated that the antibiotic resistance patterns detected in the isolates collected from Ogane-aji River Anyigba, could lead to ineffective use of antibiotics in the treatment of gastrointestinal infections in Ogane-aji community, resulting in lower alternatives for therapeutic treatments.

### **Conclusion**

The results of this study showed that Ogane-Aji River water contained multidrug resistance bacteria. Consequently, there is need to check the level of pollution of the Ogane-Aji River. Efforts also should be made to provide safe water for the Ogane-Aji River community.

### **References**

- Aarestrup, F. M., Seyfarth, A. M., Emborg, H.-D., Pedersen, K., Hendriksen, R. S. and Bager, F. (2001). Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in faecal enterococci from food animals in Denmark. *Antimicrob. Agents Chemother.* 45: 2054–2059.
- Abdul-Rasheed Abdul, Oluwatoyin, A. Igbeneghu, Adebayo, Lamikanra (2013). River Opa, a potential agent for the dissemination of multiple antibiotic resistant bacteria. 5(5): 215-221.
- Abraham, E. P., and Chain, E. (1940). An enzyme from bacteria able to destroy penicillin. *Nature.* 146: 837–839,
- Agersø, Y., and Petersen, A. (2007). The tetracycline resistance determinant Tet 39 and the sulphonamide resistance gene *sulIII* are common among resistant *Acinetobacter* spp. isolated from integrated fish farms in Thailand. *J. Antimicrob. Chemother.* 59: 23-27.
- Agunwamba, J. C. (2000). Water engineering systems. (2<sup>nd</sup> edn). Enugu: Immaculate Publication Limited, Nigeria pp. 133-139.
- Amit, A., Kumar, A., Kumar, M., Rahal, A. (2014). Multidrug resistant pathogenic *Escherichia coli* status in water source and Yamuna River in and around Mathura India. *Pak. J. Biol. Sci.* 2014: 17:540-544.
- Angulo, F. J., Nargund, V. N. & Chiller, T. C. (2004). Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among

bacteria isolated from humans and the human health consequences of such resistance. *J. Vet. Med. B Infect. Dis. Vet. Public Health* 51, 374–379.

- Bergstrom, C.T., Lo, M., and Lipsitch, M. (2004). Ecological theory suggests that antimicrobial cycling will not reduce antimicrobial resistance in hospitals. *Proc Natl. Acad. Sci. U S A.*; 101(36):13285–13290.
- Chan, M. (2011). Combat drug resistance: no action today means no cure tomorrow. World Health Organization; [cited 2011 April 14]. [http://www.who.int/mediacentre/news/statements/2011/whd\\_20110407/en/index.html](http://www.who.int/mediacentre/news/statements/2011/whd_20110407/en/index.html).
- Chessbourg, B. M. (2005). District Laboratory Practice in Tropical Countries. London Cambridge University press pp. 112-115.
- Graham, D. W., Olivares – Rieumont, S., Knapp, C. W., Lima, L., Werner, D., Bowen, E. (2011). Antibiotic resistance gene abundances associated with Waste discharges to the Almendares River near Havana, Cuba. *Environ. Sci. Technol.* 45: 418-424.
- Hawkey, P. M. (2008). The growing burden of antimicrobial resistance. *J. Antimicrob. Chemother.* 62 (4): 943–952.
- Jiang, X., Yang, H., Dettman, B. & Doyle, M. P. (2006). Analysis of faecal microbial flora for antibiotic resistance in ceftiofur-treated calves. *Foodborne Pathog. Dis.* 3, 355–365.
- Martinez, J.L. (2008). Antibiotics and antibiotic resistance genes in natural environments. *Science* 321: 365-367.
- Nester, E. W. Anderson, D. G. Robert, C. E. and Nester, M. T. (2007): Microbiology: A human perspective 5<sup>th</sup> Edition WCB/McGraw-Hill, PP:50-51.
- Nwidi, L. L., Obeh, B. Okoriye, T, Vaidosen, N. A. (2008). Assessment of water quality and prevalence of water-borne diseases in Amassoma, Niger Delta, Nigeria. *Afri. J. Biotech* 17 :2993-2997.
- Singer, R. S., Ward, M. P. & Maldonado, G. (2006). Can landscape ecology untangle the complexity of antibiotic resistance? *Nat. Rev. Microbiol.* 4, 943–952.
- Skjøl-Rasmussen, L. *et al.* (2009). Trends in occurrence of antimicrobial resistance in *Campylobacter jejuni* isolates from broiler chickens, broiler chicken meat, and human domestically acquired cases and travel associated cases in Denmark. *Int. J. Food. Microbiol.* 131, 277–279. (doi:10.1016/j.ijfoodmicro.2009.03.006)
- Smith, D. L., Harris, A. D., Johnson, J. A., Silbergeld, E. K., & Morris Jr, J. G. (2002). Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. *Proc. Natl. Acad. Sci. USA* 99: 6434–6439.
- Stephen T. Odonkor and Kennedy K. Addo (2018) Prevalence of multidrug- Resistant *Escherichia coli* isolated from drinking water sources. *International Journal of Microbiology*, article id 7204013

- Titilayo, Y. Obi, L. and Okoh, A. (2015). Occurrence of virulence gene signatures associated with diarrhoeagenic and non-diarrhoeagenic pathovars of *Escherichia coli* isolates from some selected rivers in south-western Nigeria. *BMC Microbiol.* 15-204
- United States Environmental Protection Agency. (1986). Ambient Water Quality Criteria for Bacteria-1986. EPA-440/5-84/002. Office of Water Regulations
- Wassenaar, T. M. (2005). Use of antimicrobial agents in veterinary medicine and implications for human health. *Crit. Rev. Microbiol.* 31, 155–169.
- World Health Organization (WHO). (2010). Guidelines for drinking water quality, Recommendation, Geneva p. 130.

