British Microbiology Research Journal
16(1): 1-7, 2016, Article no.BMRJ.26876
ISSN: 2231-0886, NLM ID: 101608140

SCIENCEDOMAIN *international*
www.sciencedomain.org

Incidence of Antibiotic Resistant *E. coli* Isolated from Drinking Water Sources in Ondo, Southwestern Nigeria

Olukemi Aromolaran[1*], Olubunmi Adesola-Famade[1] and Olayinka Eyitope Omoseyin[1]

[1]Department of Biological Sciences, Wesley University of Science and Technology, Ondo, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OA designed the study, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors OAF and OEO managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/26876
Editor(s):
(1) Grzegorz Cieslar, Department and Clinic of Internal Diseases, Angiology and Physical Medicine, Medical University of Silesia, Poland.
Reviewers:
(1) Wahome Caroline Nyawira, Kenyatta University, Kenya.
(2) Olaolu Oyedeji, Obafemi Awolowo University, Ile-Ife, Nigeria.
(3) Chinwe I. Chikwendu, Federal University of Technology, Owerri, Imo State, Nigeria.
(4) Valentina Urumova, Trakia University, Stara Zagora, Bulgaria.
Complete Peer review History: http://www.sciencedomain.org/review-history/15470

Original Research Article

Received 7th May 2016
Accepted 27th June 2016
Published 24th July 2016

ABSTRACT

Aim: The aim of this study was to determine the incidence and antibiotic resistant *Escherichia coli* in drinking water sources in Ondo, southwestern Nigeria.
Place and Duration of Study: Department of Biological Sciences, Wesley University of Science and Technology, Ondo between March and May, 2015.
Methodology: Fifty two (52) water samples were collected from streams and wells within and around Ondo town. Total aerobic mesophilic and coliform bacteria were determined by standard pour plate and multiple tube fermentation techniques respectively. *Escherichia coli* was isolated by cultivating on Eosin methylene blue (EMB) agar and tested for resistance to eight antibiotics by Kirby Bauer disc diffusion method.
Results: Total mesophilic count in the well and stream water samples were between 0.01 x 10^5 - 8.76 x 10^5 cfu/ml and 1.31 x 10^5 - 4.20 x 10^5 cfu/ml respectively. The MPN/100 ml of the water: well

**Corresponding author: E-mail: kemiaromolaran@gmail.com;*

(0.40 - >160) and streams (0.70 - >160). *E. coli* was confirmed present in 67.74% of all the well water and 71.43% of the entire stream. 86.11% of all the isolates were resistant to beta-lactam class of antibiotics, nitrofurantoins (11.11%), aminoglycosides (2.78%) and fluoroquinolones (2.78%). 2.78% were resistant to three classes of antibiotics (nitrofurantoins, aminoglycosides and beta-lactam). 30.56% were resistant to ampicillin, while all the isolates were sensitive to ciprofloxacin.

Conclusion: There is need for good hygiene practices and indiscriminate use of antibiotics should be discouraged, in order to reduce the release of antibiotic resistant *E. coli* to the environment.

Keywords: Wells; streams; antibiotic resistant bacteria; drinking water; Escherichia coli.

1. INTRODUCTION

More than 289 million people in Sub-Sahara Africa do not have access to safe drinking water and this poses a serious threat to public health [1]. They depend on wells and streams as their major sources of water supply. Drinking water from these untreated sources can be contaminated with pathogenic microorganisms, thereby serving as possible source of some common water-borne diseases such as diarrhea, cholera, typhoid, dysentery etc. Also, poor waste management and proximity of well water to septic tanks predisposes them to contamination [2]. It is equally important to note that unsafe drinking water sources and poor sanitation is responsible for the death of at least 1.6 m children under the age of 5 yearly and 84% of them in rural communities [3]. One sixth of the world population is sick and five million children die annually as a result of waterborne diseases [4].

The presence of *E. coli* in water is an indication of feacal contamination and presence of other pathogenic organisms [5]. Although, E. *coli* is an important flora of the human and animal intestine, it can also become important pathogens responsible for some diseases such as diarrhea in children [6,7].

Due to the exposure of human to antibiotics, *E. coli* found in the gastrointestinal tract as normal flora are discharged into water via feaces and are more likely to be resistant to antibiotics [8]. Although, most *E. coli* do not cause diseases in healthy individuals, but can transfer resistance to other strains of *E. coli* as well as other organisms within the gastrointestinal tract and to acquire resistance from other organisms [5,9]. The antimicrobial resistant strains of *E. coli* from drinking water supplies are able to transmit resistance to non-resistant *E. coli* strains. Emergence of antibiotic resistant *E. coli* strains has made it difficult to treat *E. coli* related infections. Hence, there is growing concern about resistance in *E. coli* especially in developing countries where diarrheal disease is a leading cause of illness and death [6].

The main sources of water supply for communities in Nigeria are wells and streams without any form of prior treatment. Information about antibiotic resistance in a location will help doctors to give the right prescription to patients. This study was carried out to determine the incidence and antibiotic resistant pattern of *Escherichia coli* isolated from drinking water sources within and around Ondo town, Nigeria.

2. MATERIALS AND METHODS

2.1 Description of Sample Sites

Ondo State has eighteen Local Government Areas out of which four were used for this study which are: Ile Oluji/ Oke Igbo, Odigbo, Okiti Pupa and Ondo West. The state lies between latitude 5° 45' and 7° 52'N and longitude 4° 20' and 6° 05'E. The land area is about 15,500 km^3 with estimated population of 3,440,024. People residing in these areas depend on wells and streams as their major sources of water supply [10].

2.2 Collection of Water Samples

Thirty-one (31) well water and twenty-one (21) streams water samples were aseptically collected from four communities within and around Ondo comprising Ondo town, Oke Igbo, Odigbo and Okiti Pupa between the months of March - May, 2015. Water samples were collected from sources strictly for drinking purposes. The water samples were collected in sterile glass bottles and were transported immediately to the laboratory for microbiological analysis and processed within 6 h of collection.

2.3 Bacteriological Analysis

Aerobic mesophilic bacteria count was determined by standard pour plate technique on Nutrient agar (Oxoid). Multiple tube fermentation techniques was also carried out on the water samples to determine the most probable number (MPN) of bacteria per 100 ml of water and the result was compared with the guidelines of Food and Agricultural Organization (FAO) [11]. Isolation and identification of *E. coli* was done using Eosin methylene blue (EMB) agar and incubated at 37℃ for 24 h. Greenish metallic sheen discrete colonies on EMB were picked as *E. coli* and were further identified and confirmed by biochemical tests.

2.4 Antibiotic Susceptibility Tests

E. coli isolates were subjected to antibiotics susceptibility testing by the Kirby Bauer agar disc diffusion methodology [12,13,14]. Pure colonies of *E. coli* were streaked on sterile Nutrient agar plates and incubated for 24 h at 37℃. The bacterial cells were harvested into sterile normal saline and standardized using 0.5 McFarland standard. The bacterial culture was introduced on the surface of sterile nutrient agar using sterile swab sticks. The multi-disc antibiotic was placed on the culture media aseptically, incubated at 37℃ for 24 h. The antibiotics tested and their concentrations (µg): nitrofurantoin (300 µg), ampicillin (10 µg), ceftazidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), ofloxacin (5 µg), ciprofloxacin (5 µg) and augmentin (30 µg). After incubation, the diameter of the zones of inhibition around each disc was measured. The test was done in duplicate.

3. RESULTS AND DISCUSSION

Total mesophilic count in the well and stream water samples were between 0.01 x 10^5 - 8.76 x 10^5 cfu/ml and 1.31 x 10^5 - 4.20 x 10^5 cfu/ml respectively (Table 1). The MPN/100ml of the water: well (0.40 - >160) and streams (0.70 - >160). 32.26% of all the well water sampled had >160 MPN/100ml, while 23.81% of the streams had >160 MPN/100ml. The number of coliforms in all the water sampled exceeded the WHO guideline for drinking water of zero coliform/100 ml for drinking water. The total percentage occurrence of *E. coli* in all the water sampled was 69.23%. *E. coli* was confirmed present in 21 wells (67.74%) and 15 streams (71.43%). Oyedeji et al. [15] also confirmed the presence of high number of coliforms in drinking water sources in Nigeria. He reported mean value of 1.20 x 10^4 – 7.0 x 10^4 cfu/ml and 4.9 x 10^3 - 1.20 x 10^4 cfu/ml in stream and well water respectively in Osun State, Nigeria. In a similar study in Calabar, 3.2±1.21 x 10^3 – 4.8±5.18 x 10^3 cfu/ml and 3.3±2.0 x 10^4 - 5.6±1.6 x 10^4 cfu/ml respectively were reported for total mesophilic count from well and stream water respectively [16].

The presence of *E. coli* in some of the water samples is an indication of feacal pollution. Poor sanitation is one of the leading causes of feacal contamination in drinking water. This might be due to the poor hygienic practices around the study area. High microbial load of all the water sampled from wells and streams is of great concern and a serious public health threat. Although, *E.* coli a common indicator organism which is considered to be harmless but can become opportunistic pathogen responsible for infection [6,7,17]. *E. coli* can be life threatening in immune-compromised individuals, children and elderly [2]. Therefore, it is important to enforce strict control of sewage and excreta discharge into the environment because of its health implications.

Out of a total of 52 well water and streams sampled in this study, 69.23% were positive for *E. coli,* of which 52.78% were resistant to at least one antibiotic. Fig. 1 represents the percentage resistance of *E. coli* to various antibiotics. There was more resistant *E. coli* in the stream than in the well. All the isolates from well water were susceptible to gentamicin, ciprofloxacin, ofloxacin and augmentin. 53.33% of the *E. coli* from the stream was resistant to ampicillin and ceftazidime. A study conducted in Ago-Iwoye, Nigeria was similar to this study [2]. It was reported that all the *E. coli* isolated from well water in Ago-Iwoye were resistant to amoxicillin, nitrofurantoin, cotrimoxazole but were susceptible to Ciprofloxacin and Pefloxacin. Ayandiran et al. [18] reported 40-100% resistance of gram-negative bacteria to augmentin, tetracycline, gentamicin, pefloxacin, nitrofurantoin, ciprofloxacin and amoxicillin. 33.33% of all the *E. coli* isolated from well water are resistant to at least one antibiotic, while 80% of *E. coli* from the streams were resistant to antibiotics. Overall, 30.56% of all the isolates were resistant to ampicillin, Ceftazidime (25%), cefuroxime (19.44%), nitrofurantoin (11.11%) and augmentin (11.11%). The presence of antibiotic resistant *E. coli* in drinking water could also act as a vehicle for transfer of virulent genes to some other bacteria which could result into life threating diseases [19].

Prevalence of antibiotic resistance *E. coli* to different classes of antibiotics was also determined in this study (Table 2). 86.11% of all the isolates were resistant to Beta-lactam class of antibiotics, nitrofurantoin (11.11%), aminoglycosides (2.78%) and fluoroquinolones (2.78%). Although, there is variation in the rate of *E. coli* resistance to antibiotics from one geographical location to the other [20], 26.67% of the isolated *E. coli* from stream was resistant to more than 3 antibiotics which are in agreement with the report of Efuntoye and Apanpa [2]. Many researchers have also reported the worldwide incidence of antibiotic resistant *E. coli* in drinking water especially in developing countries [21,22,23], where there are poor water supplies, poor sanitation and abuse of antibiotic [19,24,20].

Multiple antibiotic resistance (MAR) among the *E. coli* isolates from the wells and streams is presented in Table 3. None of the *E. coli* isolated from well exhibited MAR pattern, while 4 (26.67%) of the isolates from stream showed MAR pattern of AMP/CAZ/CRX/AUG, AMP/CAZ/GEN/AUG and NIT/AMP/CAZ/AUG. 11 (30.56%) of the *E. coli* were resistant to ampicillin, 9 (25%) were resistant to ceftazidime while none of the isolates was resistant to ciprofloxacin. In a similar study, *E. coli* isolated from well water samples in Nigeria were sensitive to oflaxacin, ciprofloxacin and pefloxacin but resistant to amoxicillin, nitrofurantoin and cotrimoxazole [15,20].

Table 1. Bacteriological analysis of well water and streams in Ondo

Samples	Mesophilic count cfu/ml (10^5)	Total coliform (MPN/100 ml)	Samples	Mesophilic count cfu/ml (10^5)	Total coliform (MPN/100 ml)
Wells			**Streams**		
W-A1	0.13	54.00	S-O1	1.31	2.30
W-A2	0.08	0.68	S-O2	4.20	160.00
W-A3	0.12	17.00	S-O3	2.96	>160
W-A4	0.14	>160.00	S-O4	1.83	17.00
W-A5	0.09	>160.00	S-O5	3.40	14.00
W-B1	0.20	>160.00	S-O6	2.00	92.00
W-B2	1.44	30.00	S-O7	2.30	35.00
W-B3	0.08	54.00	S-O8	2.00	>160
W-B4	0.01	35.00	S-O9	3.00	>160
W-B5	0.12	22.00	S-O10	3.94	54.00
W-C1	0.02	0.40	S-O11	3.50	0.93
W-D1	1.20	160.00	S-O12	4.00	35.00
W-E1	1.00	24.00	S-O13	3.15	92.00
W-F1	1.88	>160.00	S-I1	2.98	0.93
W-F2	0.10	28.00	S-I2	4.15	0.70
W-F3	0.15	90.00	S-I3	2.78	>160
W-F4	3.20	>160.00	S-I4	3.88	2.60
W-F5	0.12	14.00	S-I5	2.66	14.00
W-F6	0.12	90.00	S-B1	3.98	24.00
W-G1	0.05	160.00	S-B2	2.52	2.10
W-G2	0.03	0.80	S-B3	2.12	>160
W-H1	0.28	160.00			
W-I1	8.20	>160.00			
W-I2	1.70	35.00			
W-I3	0.70	30.00			
W-I4	8.76	>160.00			
W-I5	8.00	30.00			
W-J1	4.00	30.00			
W-J2	2.80	>160.00			
W-J3	4.28	>160.00			
W-J4	2.40	>160.00			

** - cfu/ml- colony forming unit per millitre; MPN – Most probable number*

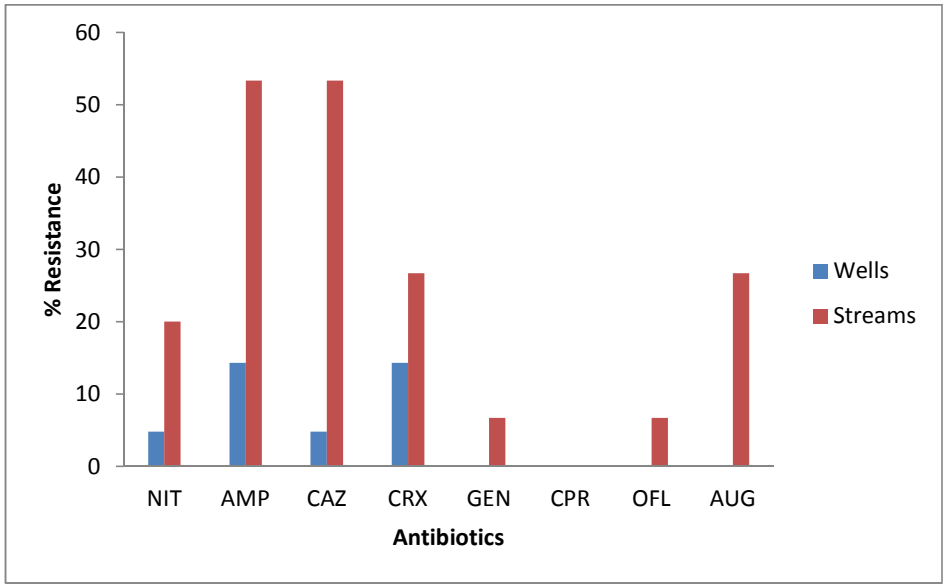

Fig. 1. Percentage resistance of *E. coli* to antibiotics

Keys: Nitrofurantoin (NIT), Ampicillin (AMP), Ceftazidime (CAZ), Cefuroxime (CRX), Gentamicin (GEN), Ciprofloxacin (CPR), Ofloxacin (OFL), Augmentin (AUG)

Table 2. Antibiotic resistance of *E. coli* from well water and streams to different classes of antibiotics

Class of antibiotic	Antibiotics	Occurrence (36)		Total	% resistance
		Well (21)	Streams (15)		
B-Lactam	Ampicillin (30 µg)	3	8	11	30.56%
	Augumentin (30 µg)	0	4	4	11.11%
	Ceftazidime	1	8	9	25.00%
	Cefuroxime	3	4	7	19.44%
Nitrofurantoins	Nitrofurantoin (300 µg)	1	3	4	11.11%
Aminoglycosides	Gentamicin (10 µg)	0	1	1	2.78%
Fluroquinolones	Ofloxacin (5 µg)	0	1	1	2.78%
	Ciprofloxacin (5 µg)	0	0	0	0

Table 3. Incidence of multiple antibiotics resistance *E. coli* isolated from wells and streams

Antibiotics	% resistance		
	Well (n= 21)	Streams (n=15)	Total (n=36)
NIT	4.76 (1)	6.67 (1)	5.56 (2)
AMP	14.29 (3)	13.33 (2)	13.89 (5)
CRX	9.52 (2)	0	5.56 (2)
CAZ	0	6.67 (1)	2.78 (1)
CAZ, CRX	4.76 (1)	13.33 (2)	8.33 (3)
AMP, CAZ	0	6.67 (1)	2.78 (1)
AMP, OFL	0	6.67 (1)	2.78 (1)
AMP, CAZ, CRX, AUG	0	13.33 (2)	5.56 (2)
AMP, CAZ, GEN, AUG	0	6.67 (1)	2.78 (1)
NIT, AMP, CAZ, AUG	0	6.67 (1)	2.78 (1)

Keys: Nitrofurantoin (NIT), Ampicillin (AMP), Ceftazidime (CAZ), Cefuroxime (CRX), Gentamicin (GEN), Ciprofloxacin (CPR), Oflaxacin (OFL), Augumentine (AUG)

4. CONCLUSION

It can be inferred from this study that majority of the people obtaining water from the study area are at the risk of water borne diseases. Also, antibiotic resistant *E. coli* is common in drinking water sources in Ondo. Ampicillin is the least effective antibiotics, followed by ceftazidime, while ciprofloxacin is the most effective antibiotic. The unguided use of antibiotics should be discouraged and good hygiene practices especially among rural dwellers should be encouraged. It is equally important that the Government should provide potable water to the people residing in the studied area, because they are at the risk of water-borne diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. WHO/UNICEF Joint Monitoring Program for water supply and sanitation. Progress on sanitation and drinking water 2010. WHO Geneva.
2. Efuntoye MO, Apanpa MO. Status of contamination and antibiotic resistance of bacteria from well water in Ago-Iwoye. Nigeria J Appl Biosci. 2010;35:2244-2250.
3. Bauwer H. Integrated water management for the 21st century: Problems and solution. Food, Agric Environ. 2003;1:136-140.
4. United State Environmental Protection Agency (USEPA). List of drinking water contaminants and their maximum contamination levels. United State Environmental Protection Guideline; 2005.
5. Bhardwaj R, Gupta A, Garg JK. Prevalence of multidrug resistance in *E. coli* strains isolated from river Yamuna, Delhi Stretch. Int J Bioassay. 2015;4: 4492-4498.
6. Coleman BL. The role of drinking water as a source of transmission of antimicrobial resistant *E. coli*. PhD Thesis, University of Toronto; 2008.
7. Galvin S, Boyle F, Hickey P, Vellinga A, Morris D, Cormican M. Enumeration and characterization of antimicrobial resistant *E. coli* bacteria in effluent from Municipal, Hospital and Secondary treatment facility sources. Appl Environ Microbiol. 2010; 76(14):4772-4779.
8. El-Zanfaly HT. Antibiotic resistant bacteria: A factor to be considered in safe drinking water. J Environ Protect and Sustain Dev. 2015;1(3):134-143
9. Oppegaard H, Steinum TM and Wasteson Y. Horizontal transfer of a multi-drug resistance plasmid between coliform bacteria of human and bovine origin in a farm environment. Appl Environ Microbiol. 2001;67(8):3732-3734.
10. Aromolaran O. Effect of *Moringa oleifera* seeds on bacterial quality of drinking water in rural communities of Ondo, Southwestern Nigeria. Ife J Sci. 2013; 15(3):631-636.
11. Food and Agriculture Organization (FAO). Chemical Analysis Manual for Food and Water 5th Edition. 1990;1:20-21.
12. Aromolaran O, Badejo OK. Efficacy of fresh leaf extract of *Spondias mombin* against some clinical bacterial isolate from typhoid patients. Asian J Trop Dis. 2014; 4(6):442-446.
13. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extract of six medicinal plants used in Southwest Nigeria unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. BMC Compl Altern Med. 2005;5:6.
14. Clinical and Laboratory Standard Institute (CLSI). Performance standard for antimicrobial susceptibility testing: Eighteenth information supplement. CLSI Document M100 – S18. Wayne: CLSI. 2008;28:1.
15. Oyedeji O, Olutiola PO, Owolabi KD and Adebayo KA. Multi-resistant feacal indicator bacteria in streams and well water of Ile-Ife city, Southwestern, Nigeria: Public health implications. J Pub Health Epidemiol. 2011;3(8):371-381.
16. Manji PL, Antai SP, Jacob IO. Incidence of *Staphylococcus aureus,* coliforms and antibiotic resistant strains of *E. coli* in rural water supplies in Calabar South Local Government. J Pub Health Epidemiol. 2012;4(9):230-237.
17. Onifade AK, Oladoja MA. Molecular epidemiology of shiga toxin-producing *E. coli* isolated from school children in Ondo State, Nigeria. British Microbiol Res J. 2015;6(6):340-347.
18. Ayandiran TA, Ayandele AA, Dahunsi SO, Ajala OO. Microbial assessment and prevalence of antibiotic resistance in polluted Oluwa River, Nigeria. The Egyp J Aqua Res. 2014;40(3):291-299.

19. Samra ZO, Naseen M, Khan SJ, Dar N, Athar MA. PCR Targeting of antibiotic resistant bacteria in public drinking water of Lahore metropolitan, Pakistan. Biomed and Environ Sci. 2009;22:458-463.
20. Onifade AK, Oladoja MA and Fadipe DO. Antibiotic sensitivity pattern of *E. coli* isolated from children of school age in Ondo State, Nigeria. Researcher. 2015; 7(2):73-76.
21. McDonald LC, Chen FJ, Lo HJ, Yin HC, Lu PL, Huang CH, et al. Emergence of reduced susceptibility and resistance to fluoroquinolones in *E. coli* in Taiwan and contribution of distinct selective pressures. Antimicrobiol Agents and Chemo. 2001; 45(11):3084-3091.
22. McKeon DM, Calabress JP, Bissonnette GK. Antibiotic resistant Gram-negative bacteria in rural ground water supplies. Wat Res. 1995;29(8):1902-1908.
23. Salvadori M, Coleman BL, Louie M, McEwen S, McGeer A. Consumption of Antimicrobial resistant *E.coli* contaminated well water: Human health impact. Application for Physicians' services Incorporated Foundation. PSI Clin Res; 2004.
24. Bello BK, Adebolu TT and Oyetayo VO. Antibiogram and plasmid profile of *E. coli* isolates in well water in Akure, southwestern Nigeria. IOSR J Pharm. 2013;3(7):30-37.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15470