International Journal of Biological Chemistry

ISSN 1819-155X

Academic Journals Inc.

www.academicjournals.com

International Journal of Biological Chemistry 1 (3): 179-183, 2007
ISSN 1819-155X
© 2007 Academic Journals Inc.

Antibiotics Assay Pattern of *Escherichia coli* Isolates from Rainwater in Ondo State, Nigeria

[1]F.C. Akharaiyi, [1]T.T. Adebolu, [2]D.E. Akpomedaye and [1]F.O. Omoya
[1]Department of Microbiology, Federal University of Technology,
P.M.B. 704, Akure, Ondo State, Nigeria
[2]Delta State University, Abraka, Delta State, Nigeria

Abstract: Twenty rainwater samples each were randomly collected from four geographical zones of Ondo State, Nigeria, which fall under a rain zone of 1200 mm annually. Eosin methylene blue agar was used to isolate *Escherichia coli* from the water, while nutrient agar was used for enumeration of total bacterial count and violet red bile lactose agar for *Coliform bacilli. Escherichia coli* isolates from the rainwater samples were subjected to antibiotic assay. Among the rainwater samples collected from each zone, Ondo State South had the highest (28.72%) *E. coli* occurrence. Also, the *E. coli* isolates from this zone were found less sensitive and resistant in most cases to the antibiotics. The average mean *E. coli* counts recovered from the rainwater samples ranged between 4.50-4. 80 (log cfu m^{-1}). This count is above the recommended value (0/100 mL) in water quality by WHO standard. The *E. coli* isolates showed various degree of sensitivity to the antibiotics potency ranging from 0->10 mm diameter inhibitory halo. Meanwhile, nitrofurantoin, augmentin and amoxylin had very low potency on the isolated *E. coli* with 0 and 25%, respectively, while others had high potency of between 75-100% on the isolates.

Key words: Geographical zone, *Coliform bacilli*, Nigeria

INTRODUCTION

The availability of quality water dictates the quality of life since water is a basic requirement of life. Despite the various sources of water supply for human use, water quality is still a luxury in developing and underdeveloped countries. Water, like air resources and its regular and plentiful supply in an uncontaminated form, is essential for the survival and health of man and most living organisms (Kumar, 2002). Since water is very essential especially for domestic purposes, the use of rainwater is a common practice in most homes in both urban and rural areas in Ondo State, Nigeria, where portable water supply is a problem. However, it had been demonstrated vividly that water sources are able to be contaminated with *Escherichia coil* (Feachem, 2001; Chao *et al*., 2004). Justus (2002) observed that the pollution of water supply with human feaces has been recognized many years as public health problems. Rather it is becoming clearer that this is not only so as sewage from crowded communities can also affect the ecological balance of the surrounding environment. The immediate environment of water sources are the major source of contamination and this include the opening surrounding (Okafor, 2005). Among bacterial isolates of diarrhea, respiratory and commensal enteric pathogens (Kunin, 1993; Sack *et al*., 1997), antibiotic resistance is increasing particularly to first-line, inexpensive and broad-spectrum antibiotics. Also, the selection and spread of resistant organisms in developing countries which can often be traced to complex socioeconomic and behavioral antecedents, contributed to the escalating problem of antibiotic resistance worldwide (Okeke *et al*., 1999). Rainwater

Corresponding Author: F.C. Akharaiyi, Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria

in its natural form supposed to be pure but the prevailing human activities such as uncontrolled waste disposal, drying up of ponds and rivers at times regulated by negative ecological distribution and the method of collection of rainwater in the area of study (6 ft or less) above ground level, could immensely contribute to the occurrence of intestinal and non-intestinal *E. coli* contamination of rainwater. Hence antibiotics are used for the treatment of both intestinal and non-intestinal *Escherichia coil* diseases, this research is therefore aimed at surveying antibiotic assay pattern of *E. coli* isolates from rainwater obtained from four different locations in Ondo State Nigeria.

MATERIALS AND METHODS

Collection of Rainwater Sample

Twenty rainwater samples each were randomly collected from the rural and urban areas of the four geographical zones (North, South, East and West) of Ondo State, Nigeria, between March and December, 2005. The rainwater samples were collected during rain into sterile polypropylene bottles from mounted stands of 6th above ground level. The bottles were allowed to fill up to the brim to trap air, tightly covered and taken to the laboratory within 2 h for analysis.

Bacteria Analysis

Nutrient agar was used for the enumeration of total viable count. With Collins *et al*. (1995) criteria, coliform count in the rain water samples was detected, where 1 mL of each sample was serially diluted to tenfold dilution and plated with violet red bile lactose agar and incubated at 37°C for 24 h. Red resultant colonies with up to 0.5 mm or larger in diameter were recognized and counted as *Coliform bacilli. Escherichia coli* in the rainwater sample was enumerated with eosine methylene blue agar, where 1 mL of each sample was plated and incubated at 37°C for 24 h. Only colonies that demonstrated green metallic sheen were recognized and identified as *E. coli*.

Antibiotic Assay

The fondiscs laboratory multor sensitivity discs (Gram negative) was used to assay the sensitivity pattern of the isolated *E. coli*. The antibiotics and its concentrations impregnated on the commercial disc arms are Augmentin (AUG) 30 mg, Amoxycllin (AMX) 25 mg, Getriazone (CRO) 30 mg, Contrimoxazole (COT) 25 g, Ciprofloxacin (CPX) 10 mg, Gentamycin (GEN) 10 mg, Nitrofurantoin (NIT) 200 mg, Ofloxacin (OFL) 54 mg, Penfloxacin (PFX) 5 mg and Tetracycline (TET) 30 mg. A concentrated 1 mL broth culture containing about 10^7-10^8 cells of the isolated *E. coli* in their log phase were pour plated with nutrient agar on sterile petri plates. The plates were allowed to solidify and stand for about 1 h, for the test organisms to be fully embedded in the medium. With a sterile forceps, the sensitivity discs were aseptically taken and carefully placed at the surface center of each seeded plates. They were incubated uninverted at 37°C for 24 h. The inhibitory halo (s) were measured with plain plastic ruler and were taken as an index of the degree of sensitivity.

RESULTS

The total bacterial mean counts in the rainwater samples ranges between 4.50-4.80 log cfu m^{-1}. Ondo State East and West zones had the least bacterial mean counts of 4.50 log cfu m^{-1} each and was highest in Ondo State South with 4.80 log cfu m^{-1} (Table 1). The rainwater samples obtained from Ondo State South also had the highest *E. coli* occurrence with 28.72% (Table 2). Meanwhile, the total percentage occurrence of *E. coil* in the rainwater collected from the four geographical zones was 100%. The sensitivity pattern of the isolated *E. coli* to the antibiotic is shown in (Table 3). The *E. coli*

Table 1: Bacterial mean viable counts obtained from the rainwater sample (log cfu m^{-1})

Zone of sample collection	Bacterial mean viable counts*
Ondo State North	4.71
Ondo State East	4.50
Ondo State West	4.50
Ondo State South	4.80

*Values are means of three replicates

Table 2: *Escherichia coli* mean counts obtained from the rainwater samples (log cfu m^{-1}) sample

Zone of sample collection	*E. coli* count (log cfu m^{-1})*	Occurrence
Ondo State North	1.48	26.24
Ondo State East	1.34	23.80
Ondo State West	0.20	21.30
Ondo State South	1.62	28.72

*Values are means of three replicates

Table 3: Antibiotic assay on the isolated *E. coli* from the rainwater

Zone of sample collection	AUG	AMX	CRO	COT	CPX	GEN	NIT	OFL	PFX	TET
Ondo State North	-	-	-	++	++	++	-	+	++	-
Ondo State East	+	+	++	++	++	++	-	++	+	-
Ondo State West	-	-	++	++	++	++	-	++	++	++
Ondo State South	-	-	+	+	++	+	-	-	-	-
Isolates to antibiotics (%)	25	25	75	100	100	100	0	75	75	75

AUG: Augmentin, AMX: Amoxicillin, CRO: Ceftriazone, COT: Contrimoxazole, CPX: Ciprofloxacin, GEN: Gentamycin, NIT: Nitrofurantoin, OFL: Ofloxacin, PFX: Pefloxacin, TET: Tetracycline, ++: Inhibitory halo >10 mm, +: Inhibitory halo<10 mm, - : No inhibition

isolated from the South zone had low response to the antibiotics potency, which denotes resistance pattern of 40%, unlike the *E. coli* isolates from East and West zone with high response of 80 and 70%, respectively to the antibiotics sensitivity (Table 3).

DISCUSSION

The danger normally associated with drinking water is contaminations by chemicals, physical and biological means generated mainly by human activities. These human activities in most cases served as air pollution. Rainwater is often subjected to such contaminations as a result of air current and the already suspended particles matters in the atmosphere. Contaminants introduced into water supply perpetuate many diseases and examples of such pathogens are *Salmonella* sp., *Shigella* sp., *Vibro cholera* and *E. coli* (Tortora *et al*., 2002). However, hence, *E. coli* has been implicated in causing human diseases such as urinary track infection (Geoffry and Charles, 1990), traveler's diarrhea (Twort *et al*., 1985), severe diarrhea in all age groups (Tortora *et al*., 2002) and many other diseases, there is need for their surveillance and possible methods of their suppression if they can not be avoided. It is very obvious that the achievement and maintenance of acceptable environment standards cannot be totally met in any community due to human activities resulting to microbial contaminations. Based on this factor, rainwater is liable to colonization with microbial cells introduced into it from the environment by windstorm or air current during rain. The quest of the people in reliably use of rainwater for domestic purposes, is that, it is less contaminated with microbial pathogens unlike surface and shallow well water which are easily contaminated from divers water runoff *E. coli* is the microbial agent usually employed as indicator of water borne pathogen (Chao *et al*., 2004). The result obtained from this study showed that the rainwater samples were contaminated with *E. coli* populations, which is far below the standard of drinking water quality (0/100 mL) as recommended by WHO (2001). Hence availability of portable water is scarce and the people depends mostly on well and rainwater, checking water borne diseases is of paramount important for the people's health safety. Meanwhile, no related work has been carried out on rainwater being consumed by the people hence

this work will add to the cautions in improving health care system in this area. The heaping up of wastes and their decomposition on the open ground surface, couple with human excrement on dump and non-dump sites and animal excrement on the street in the area of study (Akharaiyi and Omoya, 2004) could be responsible for the very high microbial load and the existence of *E. coli* contamination in the rainwater.

Despite the possibility of *E. coli* contaminating water sources, their absence in water can be avoided if sewages human and animals excrements in the environment can be controlled with much emphasis on their dangers as microbial contamination of quality water and foods.

Different sensitivity patterns were displayed by the isolated *E. coli* to the antibiotics used in this study. Contrimoxazole, Ciprofloxacin and Gentamycin, displayed high potency (100%) over the isolated *E. coli* genera than observed in the other antibiotics. Oyetao *et al.* (2007) observed that *E. coli* isolated from well water in Akure, Ondo State, Nigeria, were sensitive to norfloxacin, gentanycin, ciprofloxacin and chloramphemcol. Also, similar observation was recorded by Nazir *et al.* (2005) from *E. coil* isolates obtained from river, pond, drain and tap waters in Bangladesh. Residents of developing countries often carry antibiotic-resistant fecal commensal organisms (Calva *et al.*, 1996) been majorly due to motives for self-medication and antibiotics misuse by lay persons, health professional and unskilled practitioners.

Apparently, health people in developing countries carry potentially pathogenic antibiotic resistant organisms asymptomatically (Woolfson *et al.*, 1997). Several factors, such as urban migration with crowding and improper sewage disposal, encouraging the exchange of antibiotic-resistant organisms between people and the exchange of resistance genes among bacteria are among what that are responsible for the increase in the prevalence of resistant strains. Jawetz (1984), reported that the release of this multi-drug resistant *E. coli* through fecal materials, could eventually lead to the saturation of the environment with antibiotic resistant strains. Hence, this, however can lead to economic problem, a high knowledge of proper waste disposal and dangerous chemical substances in the environment should be acknowledge to avoid the indiscriminate spread of antibiotic resistant bacterial strains.

REFERENCES

Akharaiyi, F.C. and F.O. Omoya, 2004. Microflora of refuse dumps and site soils in five localities in Ondo State, Nigeria. Int. J. Environ. Issues, 2: 27-32.

Akharaiyi, F.C. and F.O. Omoya, 2005. Microbial survey of insect. Pest on refuse in five major towns of Ondo State, Nigeria. Global J. Environ. Sci., 4: 131-134.

Calva, J.J., J. Sifuentes-Osornio and C. Ceron, 1996. Antimicrobial resistant in fecal flora: Longitudinal community-based surveillance of children from urban Mexico. Antimicrob. Agents Chemother., 40: 1699-1702.

Chao, K.K., C.C. Chao and W.L. Chao, 2004. Evaluation of cohlert-18 for detection of coliform and *E. coli* in subtropical freshwater. Applied Environ. Microbiol., 70: 1242-1244.

Collins, C.H., P.M. Lyne and J.M. Grange, 1995. Microbiological Methods. 7th Edn., Butterworth, Heinemann Ltd.

Feachem, R.G., 2001. Drinking Water Quality. Environmental Health Engineering in Tropics. 2nd Edn., pp: 78-92.

Geoffry, R.S. and S.F. Charles, 1990. Topley and Wilson's Principles of Bacteriology, Virology and Immunity. 8th Edn., 3: 198-200.

Jawetz, E., J. Melnick and E.A. Adeberg, 1984. Review of Medical Microbiology. 16th Edn., Los Altos, Califorma, Long Medical Publication.

Justus, H.D., 2002. Microbiology Health and Hygiene. Maccmillan Press Ltd., London, pp: 112.

Kumar, H.D., 2002. Modern Concepts of Ecology. Yika Publishing House PUT Ltd., pp: 224-225.

Kunin, C.M., 1993. Resistance to antimicrobial drugs a worldwide calamity. Ann. Int. Med., 118: 557-561.

Nazir, K.H.M.N.H., M.B. Rahman, K.M. Nasiruddin, F. Akhtar, M.F.R. Khan and M.S. Islam, 2005. Antibiotic sensitivity of *Escherichia coli* isolated from water and its relation with Masmid profile analysis. Pak. J. Biol. Sci., 8: 1610-1613.

Okafor, N.O., 2005. Aquatic Water Microbiology. 4th Edn., Dimension Publishing Co. Ltd., pp: 21.

Okeke, I.N., A. Lamikanra and R. Edelman, 1999. Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotic in developing countries. Emerg. Infect. Dis., 5: 18-27.

Oyetao, V.O., F.C. Akharaiyi and M. Oghumah, 2007. Antibiotic sensitivity pattern of *Escherichia coli* isolated from water obtained from wells in Akure metropolis. Res. J. Microbiol., 2: 190-193.

Sack, R.B., M. Rahman, M. Yunus and E.H. Khan, 1997. Antimicrobial resistance in organisms causing diarrhea disease. Clin. Infect. Dis., 24: S102-105.

Tortora, J.G., R.B. Funke and L.C. Case, 2002. Microbiology: An Introduction. Media Update of Benjamin Cummings Inc., 1301 Sansome St. 7th Edn., San Fraciso, CA 9494111, pp: 712-715.

Twort, A.C., F.M. Law and W. Crowley, 1985. Water Supply. 3rd Edn., Edward Anold. World Health Organization, 2001. Water Quality Standard Health. Coma Feurtrell and Janin Bartram (Eds.), IWA Publishing Co., London UK.

Woolfson, A., R. Huebner, A. Wasas, S. Chola, P. Godfrey-Faussett and K. Klugan, 1997. Nasopharyngeal carriage of community-acquired antibiotic-resistant *Streptococcus preumoniae* in a Zamiban pediatric population. Bull. WHO., 75: 453-462.