ORIGINAL ARTILE
AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY JULY 2018 ISBN 1595-689X VOL19 No.2
AJCEM/1827 http://www.ajol.info/journals/ajcem
COPYRIGHT 2018 https://dx.doi.org/10.4314/ajcem.v19i3.6
AFR. J. CLN. EXPER. MICROBIOL. 19 (2): 195-201

RADIATION SENSITIVITY OF WATER- BORNE MULTI DRUG RESISTANT *ESCHERICHIA COLI*

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ABSTRACT

The spread of antibiotic-resistant microorganisms in the environment is recognized widely as an important public health issue, with concerns about future ability to treat infectious diseases. The main risk to public health is that the resistance genes are transferred from environmental bacteria to human pathogens. Safe water is one of the most important needs in public health in the twenty first century. Radiation sensitivity (D_{10}) is defined as the radiation dose (kGy) required to reduce the number of that microorganism by 10-fold. The aim of this paper is to determine the association between multiple antibiotic resistance and radiation sensitivity (D_{10}).Four hundred and sixty four (464) water samples were collected for assessment. *E. coli* isolation and identification were done using API 20E, and a PCR based DNA STRIP technology that allows simultaneous detection of virulence genes and confirmation of *E. coli* isolates. Antibiotic susceptibility testing was also conducted using the Kirby-bauer method. Radiation sensitivity was done using a cobalt 60 source. Sixty-three percent (63%) of the multidrug resistant *E. coli* strains recorded a multiple antibiotic resistance (MAR) index value of >0.2. The mean radiation sensitivity (D_{10}) of the *E. coli* isolates is 0.33±0.11 kGy. The study confirmed a high prevalence of multiple antibiotic resistances in *E. coli* isolates. Lastly, there is no association between multiple antibiotic resistant indexes and radiation sensitivity (D_{10}) of antibiotic resistant *E. coli.*

Keywords: Antibiotics, *Escherichia coli*, water-borne, multi drug resistant radiation, Sensitivity.

SENSIBILITÉ AU RAYONNEMENT DE L'EAU D'ESCHERICHIA COLI RÉSISTANTES AUX MÉDICAMENTS MULTIPLES

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Résumé

La propagation des micro-organismes résistants aux antibiotiques dans l'environnement est largement reconnue comme un important problème de santé publique, avec des préoccupations au sujet de la capacité à traiter les maladies infectieuses. Le principal risque pour la santé publique est que les gènes de résistance sont transférés de bactéries environnementales aux pathogènes humains. La salubrité de l'eau est un des principaux besoins en matière de santé publique au xxie siècle. Sensibilité au rayonnement (D10) est définie comme la dose de rayonnement (kGy) nécessaires pour réduire le nombre de micro-organisme par un facteur 10. Le but de cet article est de déterminer l'association entre la résistance multiple aux antibiotiques et sensibilité au rayonnement (D10).Quatre cent soixante quatre (464) des échantillons d'eau ont été prélevés à des fins d'évaluation. L'isolement et l'identification de E. coli ont été réalisés à l'aide de l'API 20E, et une bande d'ADN PCR Technologie qui permet la détection simultanée des gènes de virulence et la confirmation de l'E. coli. L'antibiogramme a également été effectuée à l'aide de la méthode Kirby-Bauer. Sensibilité au rayonnement a été fait à l'aide d'une source de cobalt 60. Soixante-trois pour cent (63 %) de la multirésistance des souches E. coli a enregistré une résistance multiple aux antibiotiques (MAR) index de >0,2.

La moyenne sensibilité au rayonnement (D10) des isolats d'E. coli le est de 0,33 0,11 kGy. L'étude a confirmé une forte prévalence de résistance multiple aux antibiotiques des isolats de *E. coli*. Enfin, il n'y a pas d'association entre plusieurs index et résistantes aux antibiotiques sensibilité au rayonnement (D10) de E. coli résistantes aux antibiotiques.
Mots clés: antibiotiques, Escherichia coli, d'origine, multi-résistante, la sensibilité.

INTRODUCTION

Radiation sensitivity D_{10} is defined as the radiation dose (kGy) required to reduce the number of that microorganism by 10-fold (one log cycle), or required to kill 90% of the total number [1] When a suspension of a microorganism is irradiated at incremental doses, the number of surviving cell forming colonies after each incremental dose may be used to construct a dose survival curve (figure 1). The relative sensitivity of different microorganisms to ionizing radiation is based on their respective D_{10} values (which is the dose required to reduce the population by 90%). Lower D_{10} values indicating greater sensitivity of the organism in question may be explained by multiple targets and/or certain repair processes being operative at low doses. Microbial cells, whether pathogenic or comprising the normal microflora of foods, exhibit differences in their responses to ionizing radiation. As ionizing radiation is a suitable method to control pathogenic bacteria in food, a large number of D_{10} values have yet been published [2]

In Ghana today very few published research are available on radiation sensitivity of microorganism. Radiation has been used to improve the safety of food substances as well as in studies involving the use of pure isolates of bacteria. Mahami *et al*., [3] sought to investigate the relative susceptibilities of planktonic versus biofilm cells of *Listeria monocytogenes* on glass to gamma radiation. Result from their study demonstrated that ionizing radiation effectively reduced the populations of both planktonic and biofilm-associated *L. monocytogenes.* The study further showed that, in contrast to chemical antimicrobial treatments, the antimicrobial efficacy of ionizing radiation is preserved or enhanced when treating biofilm associated bacteria.

In a similar study Adu Gyamfi *et al*., [4] they determined the D_{10} value (decimal reduction dose) of *Escherichia coli* in refrigerated and frozen retailed chicken. This study was done alongside the investigation of the microbiological quality of chicken, at different retail outlets. Their study observed low D_{10} values of *E. coli* especially under refrigerated conditions. This suggests susceptibility to low dose irradiation and the possibility of controlling spoilage and pathogenic microflora of fresh poultry.

However, Radiation sensitivity (D_{10}) is known to be a virulence factor. For example, the radiation sensitivity of three strains of *Escherichia coli* O157:H7 were found to increase after being induced to the antibiotic nalidixic acid [5]. However previous work, studied the radiation sensitivity only in relation to decimal reduction of bacteria but not to antibiotic resistance. Generally the effect of radiation on the antibiotic resistance of bacteria has not been extensively researched worldwide. This is particularly important when antibiotic resistance of inoculated bacteria is used as selective marker. For example, bacteria resistant to the antibiotic nalidixic acid (NalR) have recently been validated for use as a marker in studies of chemical interventions and for use in food [5]. Furthermore, the radiation sensitivity of *E. coli* isolates from water has not been determined. This is critical to further our understanding of radiation doses that will be required to eliminate water borne *E. coli* from water for safe public consumption. In addition, we do not know the relationship between multi antibiotic resistant *E. coli* and radiation sensitivity. This information is needed to provide baseline knowledge for future research in the application of radiation sensitivity to the control of multi antibiotic resistant *E. coli*. The aim of this paper is to determine the radiation sensitivity of water bourne multi-drug resistant *E. coli*

METHODOLOGY AND METHODS

Sample collection sites

After several preliminary visits to various communities in the districts, 57 sampling sites comprising six different water sources that include dams, boreholes, stream sources, rivers, canals and hand-dug wells in 27 communities were selected. Samples were taken from locations that were representative of the water sources and/or distribution networks at which water is delivered to the inhabitants and/or points of use based primarily on factors such as population and extent of usage or level of patronage of water from these sources. Most of the communities are

dominated by farmers. Each community selected had at least a borehole or a stream as the principal sources of water for the inhabitants.

Site Observation Details

Prior to water sampling, important observations were made of sanitary conditions and possible sources of contamination, both anthropogenic and natural that occur in the proximity of water bodies and/or are likely to influence water quality from all the sources sampled.

Field records for the following environmental factors were also recorded: Water clarity/turbidity (visual clarity in the water i.e. leaves, debris, algae] weather conditions (temperature, wind, rainfall) presence of animals (birds/ducks). Other comments (e.g. system problems i.e. disinfection/filtration equipment, faecal accidents)

Sample size and sampling frequency

Total of one hundred and twenty two water samples were collected for assessment. The sample collection period spanned over the two seasons in Ghana i.e. the dry and raining seasons. All water sampling and preservation procedures were performed according to Standard Methods for the examination of water and wastewater [6,7] and WHO guidelines for drinking water quality [8,9]. Sampling for bacteriological analysis was done aseptically with care, ensuring no external contamination of samples. All samples were transported to the laboratory within 2 hours.

Bacteria isolation and identification

All gram-positive organisms were identified by conventional methods, such as Gram reaction, positive catalase, Tube coagulase and Deoxyribonucleases (DNAse) test etc, whiles an API 20E kit was used to identify the gram negative organism. *E. coli* strain 25922 was used as the positive control for the E. *coli* isolates.

Anti-bacteria susceptibility testing of *E. coli*

Each of the isolates [*E. coli*] were subjected to antibiotic susceptibility testing using the Kirby bauer-method that has been standardized and evaluated by the methods of national committee for clinical laboratory standards. Isolates grown overnight on Nutrient Agar were suspended in sterile normal Saline (0.9% w/v NaCl) using a sterile wire loop until the turbidity was equivalent to 0.5 Mcfarland standards. A sterile non toxic cotton swabs dipped into the standardized innocula were used to streak the entire surface of Mueller Hinton Agar plates. The *E. coli* isolates were then tested against fourteen The data was analyzed and graphs were drawn. The inverse of the slope of each graph was taken (14)antibiotics as follows: ampicillin(10µg), Pipemidic acid (20ug), Chloramphenicol (30µg), Ciprofloxacin (5 µg), Co-trimoxazole (25 µg), Erythromycin (15 µg), Nitrofurantoin (300 µg), penicillin (10 IU), Cefuroxime (30 µg), Cefotaxime (30 µg), Nalidixic acid (30 µg), Amikacin (30 µg), Tetracycline (30 µg), and Gentamicin (10 µg), Antibiotics disks were aseptically placed using a sterile forceps, and all plates incubated (Gallenkamp England model IH-150) at 37oC for 24hrs (Mills, R., et. al. 2003). The result was interpreted using NCCLS [10].

Determination of radiation sensitivity (D_{10}) of *E. coli*

The Radiation sensitivity (D_{10}) of *E. coli* was carried out on 29 (60%) of the multidrug resistant *E. coli* isolates. Stored isolates of *E. coli* were revived by culturing them on EMB and nutrient agar media overnight at 37^{o}C in an incubator. Colonies from the overnight cultures were taken into a 100ml of peptone water. The peptone water was allowed to stand for a while for the organism to reach the stationary phase. The broth was dispensed 10ml into 7 McCarty bottles and labeled 0, 500, 600, 700, 800, 900, 1000 Gray for irradiation using the (^{60}Co source).

The exposure to the various doses of radiation was controlled from a radiation-controlled system. After irradiation with the doses mentioned above, the samples were aseptically dispensed into conical flacks containing 90 ml of peptone water. The samples were placed on a mechanical shaker for 5 minutes. The 100 ml peptone water was serially diluted into 8 other McCarty bottles containing 9ml of peptone water.

The dilutions were immediately plated in triplicates on EMB and incubated at 37^{o}C to estimate survival rates. The samples were incubated at 38^{o}C. The average of the surviving population of each dose was determined after 24 hours of incubation. This selection protocol was repeated independently three more times. Each replicate was initiated using an isolated colony derived from frozen stock of the founder.

The formula -log [N/ N_o] was used to calculate the survival curve and the dose resistance of the *E. coli* organism where N is the number of surviving *E. coli* on a plate after each dose administered and N_o is the inoculums concentration of the samples sent for irradiation.

as the dose to reduce the population of the *E. coli* organism by 1log cycle (D_{10}).

RESULTS

Table 1 shows the seasonal distribution of Multiple resistant *E. coli* isolates in the water. The number of multiple resistant *E. coli* isolated ranged between 1 and 8 in the dry season. The highest number (8) of multidrug resistant isolates in the dry reason was obtained from stream water sources, whilst the least was from river water sources (1).

TABLE 1: A SEASONAL DISTRIBUTION OF MULTIPLE RESISTANT *E. COLI* ISOLATES

Water source	No. of Multiple resistance isolates	
	Dry Season	Rainy Season
Borehole	4	6
Canal	3	1
Dam	8	6
Hand-dug well	5	4
River	1	0
Streams	7	3
N	6	6
Mean	4.67	3.33
Std Dev	2.58	2.50
S. E	1.05	1.02

SD= standard deviation, d.f= degree of freedom, Min= minimum, Max= maximum

Table 2 presents the paired t test analysis for season distribution of multiple resistances in *E. coli*. The total number of multi resistance isolates (n=6) averaged 4.67 ± 2.58 and (n=6) averaged 3.33 ±2.50 in the dry and rainy seasons respectively. There was a significance difference (t-test, and d.f. = 5, $P<0.05$) between the number of multiple antibiotic resistance *E. coli* isolated in the dry and rainy seasons.

TABLE 2: T TEST FOR THE SEASONAL DISTRIBUTION OF MULTIPLE RESISTANT *E. COLI* ISOLATES

Season	Mean±SD	Min	Max	d.f	P value
Dry	4.66 ±2.58	1	8	5	0.1576
Rainy	3.33 ±2.50	1	6	5	

SD= standard deviation, d.f= degree of freedom, Min= minimum, Max= maximum

Table 3 shows the distribution of the antibiotic resistant *E. coli* isolates and the number of antibiotic to which they were observed to be resistant. The highest number (16) of antibiotics to which *E. coli* was resistance in a single water source was observed in stream water sources and the least (1) was from borehole and river water sources. Two *E. coli* isolates were observed to be resistance to 10 different antibiotics. This was recorded in dams and stream waters sources. A summary of the resistant profiles and the kind of antibiotics to which the *E. coli* showed resistant are presented in tables 4.24 and 4.25

TABLE 3: ANTIBIOTIC RESISTANCE PROFILE (ANTIBIOGRAM) OF *E. COLI* FROM VARIOUS WATER SOURCES

Antibiotic resistant profile										Number of resist isolates
PEN	CXM	ERY	TET	CHL	PA	AMP	COT	CIP	NIT	2
PEN	CXM	ERY	TET	CHL	PA	AMP	NAL	GEN		2
PEN	CXM	ERY	TET	CHL	PA	AMK	COT			2
PEN	CXM	ERY	TET	CHL	PA	CTX				3
PEN	CXM	ERY	TET	CHL	PA					2
PEN	CXM	ERY	TET	CHL						5
PEN	CXM	ERY	TET							13
PEN	CXM	ERY								5
PEN	CXM									14
PEN										49

CHL= Chloramphenicol; COT= Co-trimoxazole; ERY= Erythromycin; NIT= Nitrofurantoin; AMP= Ampicillin; PEN= Penicillin; PA= Pipemidic acid; CIP= Ciprofloxacin; NAL= Nalidixic acid; TET = Tetracycline; CXM = Cefuroxime

Figure 2 Shows the seasonal occurrence of *E. coli* isolates with MAR index>2. The MAR Index of an isolate is defined as a/b, where a represents the number of antibiotics to which the isolate was resistant and b represents the number of antibiotics to which the isolate was subjected. It can be observed from the graph that sixteen (16) *E. coli* isolates with MAR index>2 representing 53% were obtained in the dry reason. Fourteen (14) isolates *E. coli* isolates with MAR index>2 representing 47 % were obtained in the rainy season.

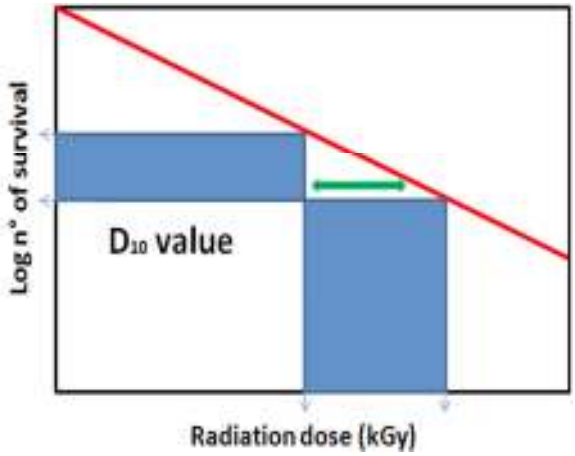

FIGURE.1: TYPICAL SURVIVAL CURVE FOR A HOMOGENEOUS MICROBIAL POPULATION

Seasonal occurrence of *E. coli* isolates with MAR index > 2

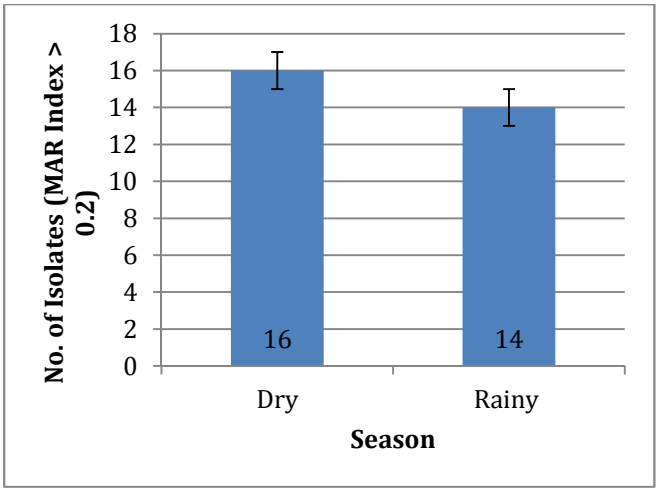

FIGURE 2: THE SEASONAL OCCURRENCE OF *E. COLI* ISOLATES WITH MAR INDEX >2

Table 4 shows the multiple antibiotic resistant indexes of *E. coli* isolates at various water sources. Borehole water sources recorded the highest MARp values of 0.9. This was followed by canal water source with a value of 0.7. The least was obtained from stream water sources and hand-dug well water sources, both recorded values of 0.4

The general statistical summary is presented first in table 5. A scatter plot of radiation sensitivity (D_{10}) and the multiple antibiotic resistances (MAR) of *E. coli* are also provided. Finally, a simple regression analysis of the association between D_{10} and multiple antibiotic resistances index of *E. coli* is presented.

TABLE 4: MULTIPLE ANTIBIOTIC RESISTANT INDEXES OF *E COLI* ISOLATES AT VARIOUS WATER SOURCES

Sampling site	Total numbers of test (isolates)	No of resistant test (resistant isolates)	MAR p
Borehole	11	10	0.9
Canal	6	4	0.7
Dam	28	14	0.5
Hand-dug well	24	9	0.4
River	2	1	0.5
Streams	26	10	0.4

MAR p = MAR index per sampling source.

TABLE 5: STATISTICAL SUMMARY OF THE RADIATION SENSITIVITY (D_{10}) OF THE MULTI-RESISTANT *E. COLI* ISOLATES

Parameter	Mean±SD	Min	Max	d.f	95.0% Confidence Interval	
					Lower Boundary	Upper Boundary
D_{10}	0.33±0.11	0.05	0.5	28	-0.133	0.79

SD= standard deviation, d.f= degree of freedom, Min= minimum, Max= maximum

Table 6 shows the descriptive statistical summary of the measures of radiation sensitivity (D_{10}) obtained from the multi-resistant *E. coli* isolates.

The radiation sensitivity (D_{10}) for 29 multidrug resistant isolates was measured. The mean radiation sensitivity (D_{10}) is 0.33±0.11 kGy. This means that average radiation doses of 0.33±0.11 kGy will be required to reduce the number of *E. coli* by 10-fold (one log cycle) or required to kill 90% of the total number [1].

TABLE 6: A SIMPLE REGRESSION ANALYSIS CORRELATION BETWEEN D_{10} AND MULTIPLE ANTIBIOTIC RESISTANCES OF *E. COLI* INDEX

	Coefficient	Standard error	Standardized coefficient	*t*	*P*
Intercept	0.270	0.043	0	6.237	0.000
Slope	0.223	0.153	0.270	1.459	.0.156
Correlation coefficient (r)=0.270, r^2=0.039					
Source	df	MS	*F*	*P*	
Regression	1	0.025	2.12	0.156	
Residual	27	0.012			

Significant at 0.05

Table 6 presents a simple regression analysis of the test of association between the radiation sensitivity (D_{10}) and the multiple antibiotic resistances (MAR) index of *E. coli*. The correlation coefficient (r) value of 0.27 indicates that there is no linear relationship between the radiation sensitivity (D_{10}) and the multiple antibiotic resistances (MAR) index of *E. coli*. The corresponding significance level of 0.156 also implies that there is no relationship or association between the radiation sensitivity (D_{10}) and the multiple antibiotic resistances (MAR) of *E. coli*. The larger insignificant P value (0.156) also suggests that changes in the predicator (multiple resistance indexes of multiple resistant *E. coli*) are not associated with the changes in the response) radiation sensitivity (D_{10})

DISCUSSION

Is there any association between antibiotic resistance and radiation sensitivity (D_{10})? The results obtained from this research have answered this question, providing new data on radiation sensitivity values for multidrug resistant *E. coli*. Ionizing radiation is a suitable method to control pathogenic bacteria in food and water, a large number of D_{10} values have been published [2,11]. Antibiotic resistance of bacteria is a commonly used selective marker. Bacteria resistance to antibiotics is believed to have an increased sensitivity to irradiation. The data obtain from the current study indicates that the multidrug resistant *E. coli* had low D_{10} values with a mean of 0.33 ± 0.11KGy. Second, test of association between D_{10} and antibiotic resistance,

was P>0.05, an indication of no association between the two parameters.

The range of *E. coli* D_{10} values obtained from the current study is consistent with previously published D_{10} values for *E. coli* [12,13]. The primary mode of action of ionizing radiation is via hydrogen and hydroxyl radical molecules resulting from the ionization of water molecules within the target organism. These radicals can disrupt membranes and interfere with the functioning of proteins, but the most significant target within the cell is DNA, where radicals are responsible for strand breakage [13]. NalR strains of *E. coli* O157:H7 and *Salmonella* have been recently shown to be more sensitive to irradiation than the NalS parent strains from which they were derived [5]. A comprehensive understanding of why a given isolate may be more or less sensitive to irradiation than related isolates of the same pathogen is yet to be formulated [12,13].
This current study did not find any association between the radiation sensitivity (D_{10}) and the multiple antibiotic resistances (MAR) of *E coli*. Furthermore, the results showed that antibiotic-resistant bacteria were preferentially associated with low D_{10} values. Thus, the study has demonstrated that ionizing radiation effectively reduces the populations of both antibiotic resistant *E. coli.* However, it is worth noting the likelihood that a survey of a larger number of isolates would result in a more linear progression of D_{10} values, that can bridge the gap seen among the statistical clusters observed among the isolates evaluated in this current study.

The implications and uses of radiation sensitivity (D_{10}) are of enough benefit to the society. First, in countries such as Austria, Czech Republic irradiation is used for drinking water disinfection [14]. Safe drinking water should not present any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages [15] (WHO, 2006). However *E. coli* in waters is a major cause of water borne diseases particularly in developing countries, where the chunk of WHO's estimated 30 000 deaths daily from water related diseases occurs [16] (Dauda, 2010). Irradiation is one of the best means of water disinfection. However, radiation sensitivity of bacteria depends on several factors [17,18]. This requires that the radiation sensitivities be evaluated for each and every organism. This study has proven that a radiation doses of 0.33±0.11 kGy could be used to disinfect *E. coli* including resistant isolates of the same in drinking water. Sachet water and bottle water producers in Ghana may have their water products disinfected for public consumption by the uses of the established recommended doses from this current study.

Finally, analysis of association between antibiotic resistance and radiation sensitivity (D_{10}) showed that that antibiotic-resistant bacteria were preferentially associated with low D_{10} values. Also a radiation dose of (0.33±0.11KGY) can be used to disinfect water contaminated with multidrug resistant *E. coli*, for safe human consumption. Furthermore, ionizing radiation effectively reduces the populations of antibiotic resistant *E. coli.*
The analysis of the findings further suggests that inactivation kinetics for controlling pathogen inactivation in food and water systems have to be estimated on the basis of specific microorganisms in food or water matrices of concern and should include further extrinsic factors. Lastly, this study has provided a model for which further research on the association between bacteria antibiotics resistances and their corresponding radiation sensitivities could be investigated.

CONFLICT OF INTEREST: Authors reports of no conflict of interest.

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