

## Effects of sub-lethal gamma irradiation on antibiotic susceptibility profile and population dynamics of *Enterococcus faecalis* and *Escherichia coli* in water

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**Abstract** Disinfection of water is important in the control of waterborne diseases. It is used to kill or inactivate microorganisms in a gradual process that involves a number of physical–chemical and biochemical processes. The effects of sub-lethal gamma irradiation on survival and resistant pattern of two indicator organisms used in assessing water quality: *Enterococcus faecalis* and *Escherichia coli* were investigated in this study. Plate count agar and disc diffusion methods were used to determine the load and antibiotics susceptibility pattern of the test organisms respectively before and after exposure to different doses of gamma irradiation in sachet water samples. Survival pattern of the two indicator organisms to gamma-ray showed a decline in the populations of the organisms with time compared to the controls (non-irradiated). The effect of the irradiation on the *E. coli* was dose dependent, it initially responded to the effects of the irradiation in the first 4 days of exposure compared to the control. On exposure to 4 and 5 Gy gamma rays a minimum population was reached on the 7<sup>th</sup> day. At  $p < 0.05$  the population of *E. coli* was significantly different from the control. The survival pattern of *Ent. faecalis* also followed a similar growth pattern. The application sub-lethal gamma irradiation did reduce the population of the isolates and also affect the antibiotic susceptibility of the isolates.

**Keywords:** *Enterococcus faecalis*, *Escherichia coli*, Gamma irradiation, Population, Water.

## INTRODUCTION

Water disinfection reduces the incidence of water-borne infections and lowers the rate of morbidity and mortality among infants and immunocompromised individuals. Chemicals like chlorine, chlorine dioxide, ozone, and irradiations are common mainly used in the treatment of drinking and wastewater (Akelah 2013; Ambulkar 2015). These chemical and physical agents kill and/or inactivate microorganisms (Akelah 2013). Irradiation has been reported to kill and inhibit the growth of pathogens like *Escherichia coli*, *Salmonella*, *Listeria*, *Shigella* and *Campylobacter jejuni* (Rothkamm 2003; Kelly 2005; Ansari 2009; Sharon Palmer 2009).

*Escherichia coli* is a Gram-negative rod of which the natural habitat is the intestinal tract of humans and animals (Farmer 2003). Due to its presence in faeces, its detection in drinking water, foods, recreational water etc. is regarded as an indicator of faecal pollution. When found in water bodies in levels exceeding one or more cells in 100 mL of water, the water is regarded as not safe for consumption. *Enterococcus faecalis*

has also been identified as an indicator of faecal contamination (Jin et al. 2004). In the European Union (EU), enterococci are used to monitor microbial quality of water. Unlike coliform, enterococci are not permitted in 100 mL and in 250 mL drinking water samples from tap and bottled water respectively (CEU 1998). The U.S. Environmental Protection Agency (EPA) has also approved enterococci in place of faecal coliform bacteria as the preferred indicator of faecal pollution and health risk in marine water (Mote et al. 2012). Both *E. coli* and *Ent. faecalis* are released through faeces of warm-blooded animals, including human beings (Wheeler et al. 2002).

Having short wavelength and high penetrating power resulting from the nuclear disintegration of certain radioactive substances such as Cobalt 60 and Cesium 137 gamma radiation is the most energetic form of electromagnetic radiation (Gross 2007). Ionizing radiation can be defined as radiation that has sufficient energy to remove electrons from atoms and molecules and to convert them to electrically-charged particles called ions (Abo-State et al. 2014). Three types of

damage in DNA are known to be induced by gamma rays and they are: single-strand breaks, double strand breaks and, nucleotide damage, which include base damage and damage in the sugar moiety (Farrag and Saleh 1996; Lodish et al. 2004; Toshihiro et al. 2006).

The production of sachet water started in the early 1990s and it's general it consumption has been very among the poor and low-income individual in Nigeria. The production, packaging and distribution of sachet water has been reported to be prone to microbial contaminated (Oyedeji et al. 2010; Onilude et al. 2013; Balogun et al. 2014). This work was aimed at evaluating the effects of gamma radiation on survival and antibiotic susceptibility pattern of two water indicator organisms: *Enterococcus faecalis* and *Escherichia coli* introduced into sachet water.

## MATERIALS AND METHODS

### Sources and standardization of test organisms

The two test bacteria were collated from the Department of Microbiology, Ekiti State University, Nigeria. The identity of the isolates was confirmed by biochemical tests. *Enterococcus faecalis* was plated on Bile aesculin azide agar (Oxoid) and incubated at 37°C for 24 h. Production of the black halo was looked for after the incubation. Gram reactions and the ability of the isolate to utilize arabinose, inulin, lactose, mannitol, raffinose, sorbitol and sucrose were also determined. *Escherichia coli* was plated on Eosine Methylene Blue Agar (LAB M, UK) and incubated at 37°C for 24 h. The plates were observed for a greenish metallic sheen. Biochemical methods which include Gram reactions, indole, Voges-Proskauer, and Methyl-Red test, utilization of citrate, fermentation of carbohydrate (arabinose, fructose, galactose, inositol, mannitol, mannose, rhamnose, ribose, sorbose and xylose) were carried out. The results were interpreted according to Holt et al. (1994).

### Preparation and introduction of indicator organisms into the water sample

The broth cultures of each of the test bacteria were centrifuged at 3000 rev/min for 5 mins after which it was decanted and washed twice with sterile distilled water and re-centrifuged at the same condition and decanted. The suspension of the washed cells was standardized according to Bauer et al. (1966).

### Inoculation of water samples for irradiation

A 500 ml of autoclave sterilized water sample was aseptically dispensed into sterile polythene sachets and approximately  $1.7 \times 10^7$  CFU/mL of the test organism was introduced. The inoculated water sample was serially diluted and plated on McConkey and Bile aesculin agars and incubated at 37°C for 24 h. to enumerate *E. coli* and *Ent. faecalis* loads respectively. The sachets of inoculated water samples were radiated by the cobalt-60 machine (gamma radiation) at a field size of 25 cm x 20 cm and depth of 2 cm with varying doses.

### Detection of bacterial load in the sample

Loads of the test organisms in both irradiated and non-irradiated water samples were determined over a period of 10 days. Daily one milliliter of the water samples was inoculated on Nutrient Agar (Oxoid) and incubated for 24 h at 37°C and the number of colonies developed on the plates was counted and recorded. This was carried out in triplicate and experiment repeated twice.

### Antibiotics susceptibility testing

One milliliter from the water samples was introduced separately into Eosin Methylene Blue and Bile aesculin azide agars to re-isolate *E. coli* and *Ent. faecalis* respectively. The colonies with distinct characteristics of the test bacteria were picked and sub-cultured. Broth of each of the organisms was standardized to 0.5 Mac-Farland standards and seeded separately on sterile Mueller-Hilton agar (Oxoid) plates. The disc diffusion method was used to assess the susceptibility of the isolates as described by the Clinical and Laboratory Standard Institute (CLSI 2012). The isolates were tested against the following different antibiotics (Abtek Biologicals Limited) with their concentrations in microgram: Ampicillin [AMP (10)], Clindamycin [CLN (2)], Clotrimazole [CLT (10)], Colistin [COL (2)], Cotrimoxazole [COT (25)], Erythromycin [ERY (5)], Fusidic acid [FUS (10)], Gentamycin [GEN (10)], Penicillin [PEN (10)], Streptomycin [STR (10)], Sulphamethaxazone [SMX (15)], Sulpholurazole [STD (200)], Tetracycline [TET (25)] and Trimethoprim [TRM (25)].

## RESULTS AND DISCUSSION

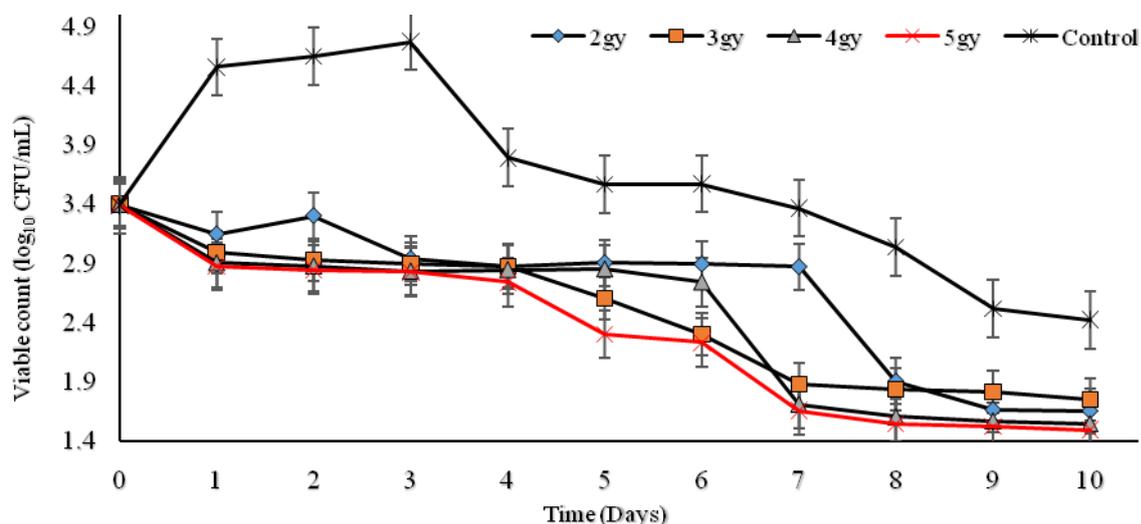
The antibiotics susceptibility pattern of *E. coli* before, and after irradiation was presented in Table 1. Gamma radiation affected *E. coli* resistance to CPL, GEN and STR. The organism was not inhibited by the antibiotics after exposure to 2, 3 and 4 gy. The zone of inhibition was slightly shown after exposure to 5 gy. While *Ent. faecalis* had a lower zone of inhibition when exposed to CLN, ERY, FUS, and GEN. On the other hand, SMX and TRM had a higher inhibitory effect on the isolates after exposure to 5 gy. His trend is similar to the report of Meckes (1982).

The results showed *Ent. faecalis* to be resistant to all the antibiotics tested after exposure to a dose rates of 2 gy and 3 gy (Table 2). At dose rate of 5 gy, the organism was sensitive to all the antibiotics except penicillin. After irradiation *Ent. faecalis* produced a lower susceptibility to most of the antibiotics. This is at variance with the report of Eman (2003) who reported that enteric bacteria were susceptible to tetracycline and ampicillin after irradiation. In a similar trend, Yahia et al. (2015) reported that gamma irradiation increased the sensitivity of *Salmonella* to different antibiotics.

**Table 1** Antibiotic susceptibility profile of *E. coli* before and after irradiation (zone of inhibition in mm)

Antibiotics	Before	After irradiation (gy)			
		2	3	4	5
AMP	0	0	0	0	0
CLT	0	0	0	0	0
COT	0	0	0	0	0
CPL	9	0	0	0	1.3±1.0
GEN	11.3±2.3	0	0	0	1.7±1.0
STD	0	0	0	0	0
STR	10.2±1.6	0	0	0	0
TET	0	0	0	0	1.2±0.3

COL- Colistin, GEN- Gentamycin, STR- Streptomycin, STD- Sulpholurazone, TET- Tetracycline, COT- Cotrimoxazole, AMP- Ampicillin, CLT- Clotrimazole

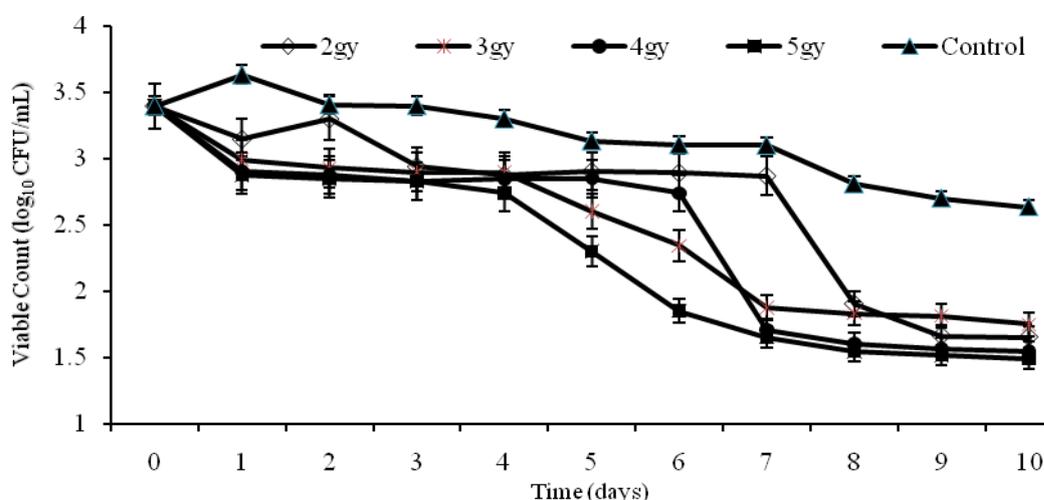


**Fig 1** Survival rate of *E. coli* recovered from non-radiated sachet water

**Table 2** Antibiotic susceptibility profile of *Enterococcus faecalis* before and after irradiation (zone of inhibition in mm)

Antibiotics	Before	After irradiation (gy)			
		2	3	4	5
CLN	6.1±2.5	0.5±0.1	0	0	2.5±1.2
ERY	4.6±1.0	0	0	0	2.2±1.0
FUS	9.3±1.4	1.5±1.0	0	1.8±0.2	3.6±1.4
GEN	8.4±2.0	1.3±0.2	0	0	1.9±0.2
PEN	0	0	0	0	0
SMX	0	0	0	0	2.7±1.0
TET	2.6±1.4	0	1.9±0.1	1.5±0.2	2.9±1.0
TRM	0	0	0	2.2±0.1	2.1±1.0

GEN- Gentamycin, FUS- Fusidic acid, TRM- Trimethoprim, SMX- Sulphamethaxazole, TET- Tetracycline, PEN- Penicillin, ERY- Erythromycin, CLN- Clindamycin

**Fig 2** Survival rate of *Ent. faecalis* recovered from non-radiated sachet water

Abojassim et al. (2016) reported the presence of a significant positive correlation between gamma irradiation and inhibition of different types of pathogenic bacteria. The reduction in bacterial susceptibility may be due to chromosomal exchange or mutation (Johnson et al. 2006; Amyes 2007; Mundy et al. 2000). Also, water serves as possible vectors of strain transmission to human intestinal flora (Witte 2000; Salyers 2004). After irradiation, the viability for both *E. coli* and *Ent. faecalis* decreased at a dose-dependent rate compared with the control. This agreed with the result of Tuncayciyumus et al. (2008) who reported low bacterial load in meatballs challenged with *E. coli* and *Ent. faecalis* after gamma radiation. Gamma radiation induces the DNA by interfering with the genetic material causing the variety of changes and breaks in DNA strands (Rothkamm 2003; Lodish et al. 2004; Prescott et al. 2008) thereby making the organisms either resistant or susceptible after irradiation (Lodish et al. 2004).

The ability of the ray to cause permeability of ionic channels in the bacterial membrane, damage of DNA and impairment of efflux pump systems have been reported to be mechanisms of action of gamma ray (Berrier et al. 1993; Farrag and Saleh 1996; Galvanoskis et al. 1999; Pouget et al. 1999; Yahia et al. 2015). Figures 1 and 2 show the survival time of *E. coli* and *Ent. faecalis* respectively. After irradiation, the viability of both *E. coli* and *Ent. faecalis* decreased gradually as time increased. The effect of the irradiation on the *E. coli* and *Ent. faecalis* was dose-dependent. Abo-State et al. (2014) reported that the pathogenic bacteria strains isolated from water showed no growth after exposure to irradiation. The total aerobic mesophilic count has been reported to be lower after exposure to gamma-ray (Pouget et al. 2002; Zafer and Gurbuz 2007).

## CONCLUSION

Gamma radiation reduced the population of the test organisms in the water sample but does not positively affect their susceptibility to antibiotics. The exposure of the sachet water to irradiation before the final packaging will further reduce the bacterial contamination and subsequently water-borne infections.

## ACKNOWLEDGEMENT

The authors acknowledge the contribution of Mr. S.S. Ajayi of the Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria for his technical assistance.

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