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Effect of Gamma Irradiation on Escherichia coli Isolated from Milk and Dairy Products

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ABSTRACT

Escherichia coli (E. coli) is one of the pathogens that can cause foodborne diseases and can cause serious health problems. In the current study, a total of 100 random samples of raw milk (n= 50), cheese (n= 25), and yogurt (n= 25) were collected from different shops and supermarkets at Menoufia Governorate, Egypt. *E. coli* was isolated from 32 examined samples and subjected to biochemical and serological tests for identification. Ten out of 32 isolates tested to antimicrobial susceptibility, were highly resistant for penicillin, chloramphenicol, co-trimoxazole (sulpha, trimethoprim) (100%), followed by cotrimoxazole25 (90%), ampicillin (70%) and cefotaxime (70%). The examined isolates were highly sensitive to gentamicin (90%), linezolid (90%) and kanamycin (80%), and moderately sensitive to streptomycin (60%). Bacteria employ several methods to resist antibiotics, primarily by altering their structure or function to prevent the drug from working effectively. Gamma irradiation is a non-thermal processing technology that is used as a method of preserving foods in several countries and can be used to eliminate food-borne pathogens. Three multidrug resistance (MDR) *E. coli* isolates were exposed to gamma irradiation at doses of 0, 0.5, 1, 2, 4, 8 KGy, then tested for the effect of Gamma Irradiation (antimicrobial susceptibility, bacterial cell mass, total *E. coli* count, antimicrobial activity test of *E. coli* and PCR). Gamma Irradiation was found to have a significant effect that led to decreased total *E. coli* count, bacterial cell mass and changed resistance of some strains tested for *dfr*A and *floR* genes by PCR.

1. INTRODUCTION

Food borne pathogens in raw milk may increase the threat of ingestion and transmission of food borne pathogens and ingestion of harmful toxins. Huge numbers of microbes can get access to milk and various dairy products including E. coli which is an indicator of fecal contamination, constituting a public health hazard. [1]

E. coli is a Gram-negative, facultative anaerobic, rod-shaped bacterium that belong to the family Enterobacteriaceae. E. coli is one of the primary gastrointestinal occupants. Most E. coli are commensal, however some of them may be dangerous and cause diseases all over the world. [2]

Being rich in proteins, lipids and sugars, milk is an example of ideal culture medium for E. coli pathogen. This pathogen has been associated with milk and dairy products.[3]

There are different strains of *E. coli* present in nature and usually found in the intestines of healthy humans and animals. There are some strains of *E. coli* that are pathogenic and can cause food-borne disease.[4]. It can produce a clinical illness characterized by an acute grossly bloody diarrhea that is accompanied by severe, crampy abdominal pain .A few patients go on to develop hemolytic uremic syndrome or thrombotic thrombocytopenic purpura.[5, 6]

Based on the mechanism of illness, *E. coli* strains can be divided into six classes called pathotypes. *E. coli* strains that are enteropathogenic (EPEC), enterotoxigenic

(ETEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC), and enteroaggregative (EAEC). Vero Toxin-Producing *E. coli*, Shiga-toxigenic *E. coli*, and Enterohaemorrhagic *E. coli* are names given to the *E. coli* strains that manufacture six toxins [7]

E. coli serotyping using O (lipopolysaccharide), K (capsular), and H (H, flagellar) antigens is a method to classify different strains of *E. coli*. These antigens are surface structures that can be identified through specific antibodies, allowing for the differentiation of strains that might cause different diseases or have varying virulence factors.[8]

Irradiation is a novel, non-thermal processing technology for food. Using of ionizing irradiation (gamma irradiation) can cause changes in cells and destroy microorganisms.[9]

Food irradiation prolongs shelf life better than thermal processing and eliminates the need for artificial preservatives, preserving food's nutritional value. [10]

The approval of gamma irradiation to eliminate food-borne pathogens[9, 11–14] makes it appropriate to determine the effect that irradiation treatments would have on *E. coli* .

Therefore, this prospective study aims to isolation and identification of *E. coli* and evaluates the efficiency of Gamma irradiation on *E. coli* isolated from milk and dairy products.

2. MATERIALS AND METHODS

2.1 Samples collection

A total of 100 random samples of raw milk (50 samples), cheese (25 samples), and yogurt (25 samples) were collected from various shops and supermarkets in Menoufia Governorate, Egypt. All collected samples were aseptically transferred to the laboratory in an insulated ice box and immediately subjected to microbiological identification.

2.2 Isolation and identification

Twenty-five ml or g. from each collected and prepared sample were added to sterilized flask containing 225 ml of sterile Buffered Peptone Water (BPW; Oxoid, New Hampshire, UK) , maintained for 1 h at 25 ◦C. From each homogenate, 1.0 mL was aseptically inoculated into 9.0 mL of sterile nutrient broth (Oxoid, New Hampshire, UK) and incubated at 37˚C for 24 hrs. It was used for the growth and propagation of isolates before platting under aerobic condition according to ISO 7251:2005.[15] Then streak a loopful from the prepared samples into MacConkey's agar plates (Oxoid, New Hampshire, UK) and incubate for 24 hours at 37ºC. Suspected lactose fermented colonies were picked up and streaked on Eosin methylene blue media (EMB) (Oxoid, New Hampshire, UK) then incubated for another 24-48 hours at 37ºC, suspected colonies were subjected to biochemical tests.[16] and Congo red agar medium. Each isolate was streaked on a sterile separate plate and kept at 37°C for 24 hrs. The cultures were kept at room temperature for 48 hours. After 48 hours in room temperature.[17]

2.3 Microscopical examination

Smears from suspected pure colonies were stained with Gram- stain and examined microscopically. [18]

2.4 Biochemical identification

Identification of *E. coli* isolates was confirmed biochemically by sugar fermentation tests, indol, methyl red, Voges-Proskaur, citrate utilization, H2S production, urease, catalase, oxidase, and nitrate reduction tests.[18]

2.5 Serological identification

By performing a slide agglutination test with conventional polyvalent and monovalent *E. coli* antisera (Denka Seiken-Co., Ltd., Tokyo, Japan), at Animal Health Research Institute, Dokki, Egypt. *E. coli* isolates were identified serologically .[19]

2.6 Antimicrobial susceptibility test

Antimicrobial susceptibility test was performed on *E.coli* isolates using the Kirby-Bauer disc diffusion technique. Mueller Hinton Agar (MHA) (Oxoid, New Hampshire, UK) was prepared as the manufacturer directed and cooled to 45-50°C before pouring into plates. A broth culture of *E. coli* isolates aged 18-24 hours was standardized by diluting to 0.5 McFarland's standard and the results were

interpreted as sensitive, intermediate, or resistant according to Clinical and Laboratory Standards Institute guidelines for (CLSI, 2023) [20]

The obtained bacterial isolates were tested in vitro for their susceptibility to the following antimicrobial discs (Oxoid, New Hampshire, UK): penicillin (p) 10 IU, kanamycin (K) 30µg, streptomycin (S) 10µg, chloramphenicol (C) 30µg, cefotaxime(Ct)30µg, ampicillin 10 (Am) 10µg, linezolid (lz) 30µg, gentamicin (Gen) 30µg, co-trimoxazole (sulpha,trimethoprim) (cot) 25µg .[20]

2.7 Gamma Irradiation

Three Isolates were exposed to gamma irradiation at doses of 0, 0.5, 1, 2, 4, 8 kGy. The process of irradiation was carried out at the Nuclear Research Center, Atomic Energy Authority, Inshas, Egypt. The facility used was a 60Co gamma chamber, MC20, Russia. Irradiation was performed using 60Co gamma rays at a dose rate of 0.68409 kGy/h at the time of the experiment. [21]

2.8 Total *E. coli* count

One ml from the serial dilution of the irradiated isolates and control was cultured on TBX agar by pour plating method and incubated at 44º C for 24 hours. Morphologically typical colonies (blue colony) were counted.[22]

2.9 Bacterial cell mass (biomass) using dry weight method with filter paper:

Dry weight measurements were used for estimating microbial biomass. The culture is harvested by centrifugation at ~10,000 rpm for 10 min and then filtration by a pre-weighed and labeled dry filter paper, washed, dried at 80°C to constant weight, and weighed on sensitive balance to obtain the biomass of the *E. coli* isolates.[23]

2.10 Molecular examination

A selected *E. coli* isolate (sample 2) that gamma irradiation changed its antimicrobial susceptibility for chloramphenicol and co-Trimoxazole from resistant to sensitive, showed best growth according to total *E. coli* count and biomass through different gamma irradiation doses.

The selected isolate was examined for detection of resistance genes: Florfenicol (*flo*R gene) and Dihydrofolate reductase enzyme *(dfr*A gene) before and after gamma irradiation following the QIAamp® DNA Mini Kit instructions (Qiagen Inc., Valencia, CA, USA), Emerald Amp GT PCR mastermix (2x premix) (Takara, Japan) was prepared as shown in table (1). The PCR program used the primers shown in Table (2) and running conditions as described in Table (3). PCR products were separated by electrophoresis in 1.5% agarose gel with 0.5µg/ml ethidium bromide for 30 min. PCR amplification bands were visualized by transilluminator in UV cabinet[24]. A gel documentation system photographed the gel, and the data was analyzed using computer software.

Table (1): Preparation of PCR Master Mix according to Emerald Amp GT PCR mastermix (Takara) Code No.RR310A kit:

Component	Volume/reaction
Emerald Amp GT PCR mastermix (2x premix)	**12.5µl**
PCR grade water	**5.5 µl**
Forward primer(20 pmol)	**1 µl**
Reverse primer (20 pmol)	**1 µl**
Template DNA	**5 µl**
Total	**25 µl**

Table (2): Oligonucleotide primers sequences from Metabion (Germany) used for detection *of E. coli* resistance genes:

Primer	Sequence	Amplified product	Reference
***flo*R**	F 5′-TTTGGWCCGCTMTCRGAC-3′	494 bp	Doublet *et al*., 2003
	R 5′-SGAGAARAAGACGAAGAAG-3′		
***dfr*A**	F 5′-TGGTAGCTATATCGAAGAATGGAGT-3′	425 bp	Grape *et al*., 2007
	R 5′-TATGTTAGAGGCGAAGTCTTGGGTA-3′		

Table (3): Cycling conditions of the different primers during cPCR

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
*flo*R	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec	72˚C 45 sec	35	72˚C 10 min.
*dfr*A	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec	72˚C 45 sec	35	72˚C 10 in.

2.11 Antimicrobial activity of E. coli against Other Microorganisms

To evaluate whether *E. coli* produces antimicrobial compounds capable of inhibiting the growth of other bacterial species, such as *Staphylococcus aureus* (*S. aureus*) ATCCQ 6538 TM(Animal Health Research Institute, Shebin El Kom, Egypt) and *Klebsiella pneumoniae* (*K. pneumoniae*) , spot-on lawn antimicrobial assay was performed with slight modifications.[25]

A nutrient agar plate was first uniformly seeded with a fresh overnight culture of *E. coli*. The plate was incubated at 37°C for 18–24 hours to allow potential antimicrobial compound production (e.g., colicins). Following incubation, 10 µL of an overnight culture of the test organism (*S. aureus* and *K. pneumoniae*) was carefully spotted onto the *E. coli*-seeded plate surface. Plates were then reincubated at 37°C for an additional 24 hours. Zones of inhibition or clearance around the test organism spots were observed visually and recorded as evidence of antagonistic or antimicrobial activity by *E. coli*.

3. RESULTS

3.1 Incidence of *E. coli* isolated from examined samples.

A total of 32 isolates of *E. coli* were recovered from 100 samples and represented as 20/50(40%), 10/25 (40%), 2/25 (8%) from of raw milk, cheese, yogurt, respectively.

3.2Identification of *E. coli*:

The isolated strains were cultured, into MacConkey Agar, revealed smooth, circular pink colonies with spreading growth. On EMB: showed (metallic green colonies) **(Figure 1).** On TBX: blue or blue green colonies **(Figure 2).**

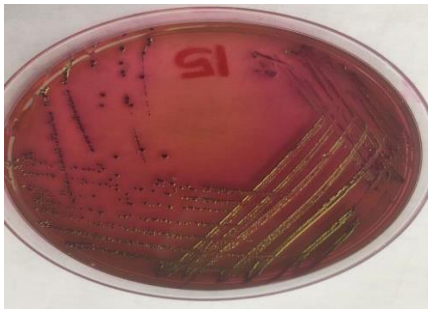

Fig (1) Colonies of *E.coli* on EMB Agar media (High metallic green sheen producing E. coli colonies)

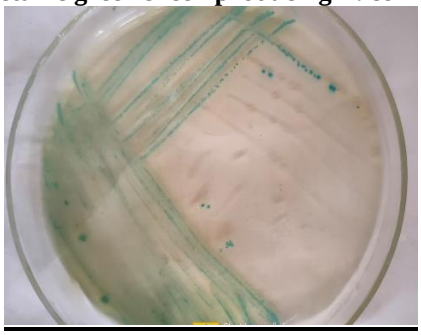

Fig (2) Colonies of E.coli on TBX Agar media (Blue or blue green colonies)

3.3 Microscopical examination

E.coli were confirmed through Gram staining, *E.coli* is motile facultative anaerobic Gram-negative Pink to red Shape Rod-shaped (bacilli) Arrangement, Single or short chains.

3.4 Biochemical identification

Biochemically, Catalase + ve Simmons's citrate –ve TSI A/A+ gas Indole Production + ve Nitrate Reduction + ve Urease –ve Voges Proskaur –ve Methyl Red + ve Glucose fermentation+ ve Lactose fermentation + ve **Table (4)** as shown **(Figure 3)**.

Fig (3) Biochemical identification of E.coli

Table (4): Biochemical identification of *E.coli*:

Characteristics	Result	Characteristics	Result
Oxidase	- ve	Methyl red	+ ve
Catalase	+ ve	Vogasproskauer (VP) tests	- ve
Indole test	+ ve	Citrate utilization	- ve
Hydrogen sulphide(TSI)	- ve	Urease test	- ve
Glucose fermentation	+ ve	Lactose fermentation	+ ve

Antimicrobial disc	Code	Disk concentration	Sensitive		Intermediate		Resistant		AA
			No.	%	No.	%	No.	%	
Penicillin	p	10 IU	0	0.0	0	0.0	10	100	**R**
Kanamycin	K	30µg	8	80	1	10	1	10	**S**
Streptomycin	S	10µg	6	60	0	0.0	4	40	**S**
Chloramphenicol	C	30µg	0	0.0	0	0.0	10	100	**R**
Cefotaxime	Ct	30µg	3	30	0	0.0	7	70	**R**
Ampicillin	Am	10µg	2	20	1	10	7	70	**R**
Linezolid	lz	30µg	9	90	0	0.0	1	10	**s**
Gentamicin	Gen	30µg	9	90	0	0.0	1	10	**s**
Co-Trimoxazole (Sulpha,Trimethoprim)	Cot	25µg	0	0.0	0	0.0	10	100	**R**
Cotrimoxazole	Sxt25	25µg	0	0.0	1	0.0	9	90	**R**

Table (6): In vitro anti-microbial sensitivity test for isolated pathogenic E. coli

3.5 Activity of pathogenic E.coli on Congo red medium

E. coli isolates were tested for their pathogenicity by culturing on Congo red medium. The results revealed that 10 (10%) out of total 100 collected samples appear as dark red colonies as showed in **(Figure 4)**.

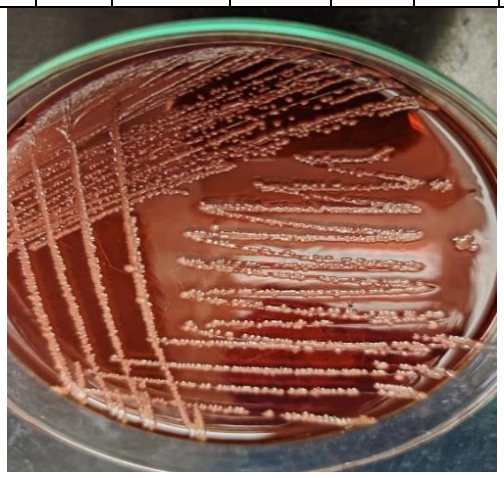

Fig (4) pathogenic *E.coli* on Congo red medium (E.coli appear as dark red colonies on Congo red medium)

3.6 Serological identification

Serological identification of 10 pathogenic *E. coli* strains shown in Table **(5)**

Table (5): Serological identification of 10 pathogenic *E. coli* strain

Serodiagnosis *E.coli*	No. of positive	Percent %
O44:K74	3	30
O78:K80	2	20
O114:K90	1	10
O125:H21	2	20
O111:H2	2	20
Total	10	100

3.7Antimicrobial susceptibility test results

Based on CLSI[20] breakpoints, **Table (6)** Results of antimicrobial sensitivity test on 10 isolates of *E.coli* recovered from raw milk, cheese, yogurt were highly resistant for Penicillin, chloramphenicol , Co-trimoxazole (sulpha,trimethoprim) (100%) , followed by Cotrimoxazole 25(90%), Ampicillin (70%), cefotaxime(70%) and highly sensitive for gentamicin(90%), linezolid (90%), kanamycin (80%), and Streptomycin (60%). **(Figure 5).**

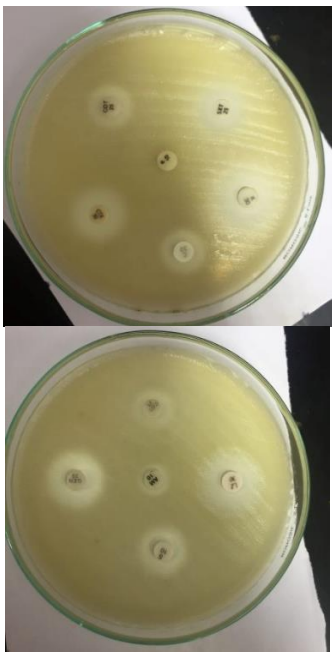

Fig (5) Antibiotic susceptibility test of *E. coli*

3.8 Antimicrobial susceptibility test after gamma irradiation

Antimicrobial susceptibility test after gamma irradiation at doses (0.5, 1, 2, and 4) kGy was done *and growth inhibition zone diameter was measured in mm.* **Table (7) (Figure 6).**

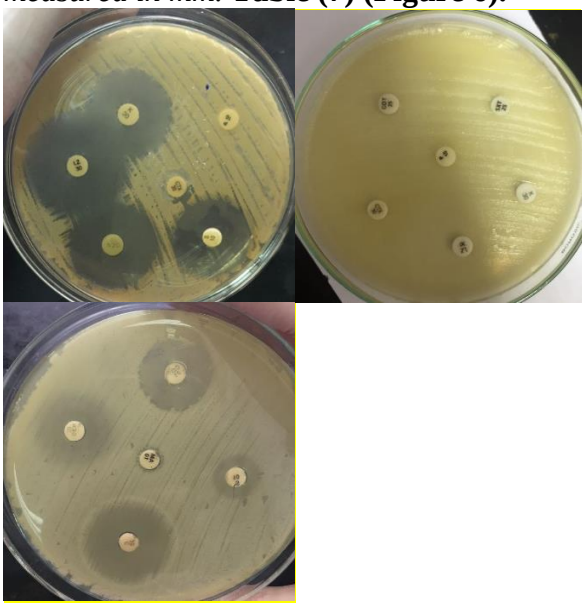

Fig (6): antimicrobial susceptibility test of E. coli after gamma irradiation.

Table (7): antimicrobial susceptibility test after gamma irradiation at doses (0.5, 1, 2, and 4) kGy of E. coil and growth inhibition zone diameter in mm.

3.9 Total E. coli count

Total E. coli count (log10 CFU/ml) at different Gamma Doses (kGy) for three samples at two dilutions (10^{-2} and 10^{-4}) was done. E. coli count decreases with increasing gamma radiation .At 8 kGy, all counts fall below detectable levels (<10^2).The reduction is consistent across all samples and dilutions, showing the effectiveness of gamma radiation. **Table (8) (Figure 7).**

Gamma Dose (kGy)	Sample 1 (log CFU/ml)		Sample 2 (log CFU/ml)		Sample 3 (log CFU/ml)	
	10^{-2}	10^{-4}	10^{-2}	10^{-4}	10^{-2}	10^{-4}
0 (Control)	5.63	7.49	5.68	7.5	5.61	7.49
0.5	5.64	7.48	5.69	7.5	5.62	7.49
1	5.37	6.99	5.62	7.48	5.31	6.92
2	5.03	6.53	5.25	6.79	4.48	6
4	4.48	6	4.98	6.18	4.4	5.6
8	<10^2 (ND)	<10^2 (ND)	<10^2 (ND)	<10^2 (ND)	<10^2 (ND)	<10^2 (ND)

Table (8): Total E. coli count by (log10 (CFU/ml))

<10^2 below detectable level

Antimicrobial disc	Isolate												
	1				2					3			
	C	0.5kGy	1KGy	2kGy	C	0.5kGy	1KGy	2kGy	4kGy	C	0.5kGy	1KGy	2kGy
Penicillin(p)	R -	R -	R -	R -	R -	R -	R -	R -	R -	R -	R -	R -	R -
Kanamycin (K30)	S 18	S 18	S 20	S 22	S 20	S 20	S 22	S 22	S 24	S 20	S 22	S 24	S 26
Streptomycin (S)	S 16	S 16	S 18	S 20	S 16	S 16	S 18	S 20	S 22	S 18	S 16	S 18	S 20
Chloramphenicol (C30)	R 8	R 12	S 18	S 20	R 8	R 10	R 12	S 18	S 20	R 8	R 10	R 10	R 12
Co-Trimoxazole (Cot25) (Sulpha,Trimethoprim)	R 8	R 10	S 16	S 20	R 6	R 8	R 10	S 16	S 22	R 8	R 6	R 8	R 10
Cefotaxime (Ctx30)	R 10	R 12	R 12	R 16	R 10	R 12	R 14	R 16	R 22	S 26	S 28	S 28	S 30
Ampicillin (Am10)	R 6	R 8	R 10	R 12	R 10	R 8	R 10	R 10	R 12	S 18	S 18	S 18	S 20
Linezolid (lz)	S 30	S 32	S 32	S 34	S 24	S 24	S 26	S 28	S 30	S 28	S 30	S 30	S 32
Gentamicin (Gen30)	S 18	S 20	S 20	S 22	S 18	S 22	S 22	S 24	S 24	S 20	S 20	S 22	S 22
Cotrimoxazole (SXT25)	R 8	R 8	R 10	R 10	R 6	R 8	R 10	S 16	S 20	R 6	R 8	R 8	R 10

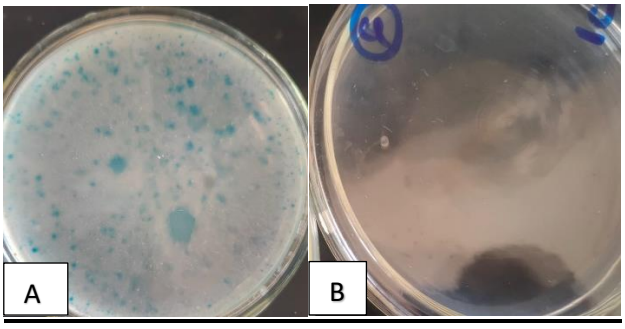

Fig (7) Total E. coli count on TBX Media, (A) represent total E.coli count at 0.5 kGy, (B) represent total E.coli count at 8 kGy.

3.10 Bacterial cell mass (biomass) test

Comparison of three isolate for estimating the dried mass of *E.coli* before and after gamma irradiation **Table (9).**

A reduction in biomass is observed with increasing irradiation dose, and no detectable biomass was recorded at 8 kGy in all three samples, indicating complete bacterial inactivation**.**

Table (9): Biomass Test

Sample	Wt. of membrane Filter blank	After 12hr (70°c)	*Bacteria/gm*
1(C)	0.9900	1.0052	0.0152
1 (0.5KG)	0.9877	1.003	0.0153
1 (1 KG)	0.9887	1.0017	0.013
1 (2 KG)	0.9959	0.997	0.0011
1 (4 KG)	0.9958	0.9958	0
1 (8 KG)	0.9891	0.9891	0
2(C)	0.9892	1.0122	0.023
2 (0.5KG)	0.9950	1.02	0.025
2 (1 KG)	0.9918	1.0068	0.015
2 (2 KG)	0.9898	0.9988	0.009
2 (4 KG)	0.9906	0.9936	0.003
2 (8 KG)	0.9913	0.9913	0
3(C)	0.9911	1.009	0.0179
3 (0.5KG)	0.9923	1.0041	0.0118
3 (1 KG)	0.9905	0.9996	0.0091
3 (2 KG)	0.9909	0.9936	0.003
3 (4 KG)	0.9940	0.9940	0
3 (8 KG)	0.9956	0.9956	0

3.11 PCR Results

Detection of resistant genes of *E. coli*. PCR using primers fragments for detection of two resistant genes, these genes were *dfr*A and *flo*R genes of *E.coli*. It was applied on one isolates of *E.coli* before and after gamma irradiation. It was applied for detection *dfr*A and *flo*R genes of *E.coli* that isolate (1) before gamma irradiation and (2) after gamma irradiation. **Table (10) (Figure 8).**

Table (10): PCR Results

Sample	*flo*R	*dfr*A
1	+	+
2	-	-

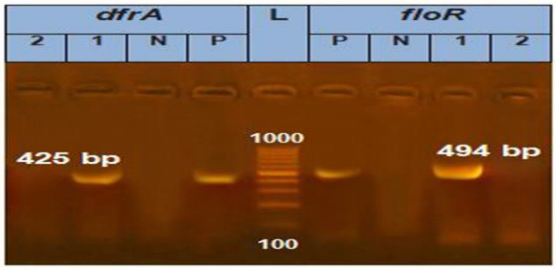

Fig (8) Agarose gel electrophoresis pattern of PCR for detection of *dfr*A and *flo*R genes of *E.coli* at 425 bp and 494 bp.

L: Ladder from 100 bp to 1000 bp.

P: Positive control: *E. coli* ATCC 25922.

N: Negative control: Field isolate that were tested and confirmed to be negative by PCR for the related genes in R.L.Q.P

Lane 1 positive amplification of *dfr*A and *flo*R genes.

Lane 2 Negative amplification of *dfr*A and *flo*R genes.

3.12Antimicrobial Activity *of E. coli* against *other microorganisms such as Staphylococcus aureus* and *Klebsiella pneumoniae*[26]

Zones of inhibition or clearance around the test organism spots were observed and recorded as evidence of antagonistic or antimicrobial activity by *E. coli*. *E. coli* inhibited the growth of *Klebsiella* after exposure to 4 kGy of gamma irradiation. **(Figure 9).**

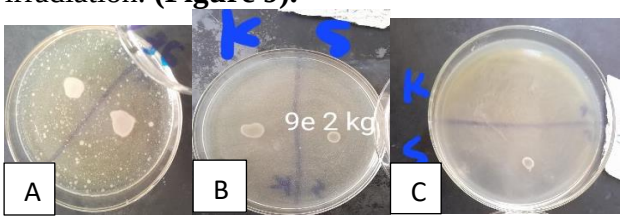

Fig (9): Antimicrobial Activity of *E. coli* against *Staphylococcus aureus* and *Klebsiella pneumoniae* before and after exposure to gamma Irradiation. (A) represent activity of control isolate (before exposure to gamma irradiation). (B) represent activity after exposure to 2 kGy of Gamma irradiation, (C) represent activity after exposure to 4 kGy of Gamma irradiation , *E. coli* inhibited the growth of *Klebsiella* .

4. DISCUSSION

The incidence of foodborne diseases has increased over the past years and resulted in major public health problem globally [27]. In this study 20 isolates of *E. coli* (40 %) were obtained from 50 samples of raw milk. These

results were agreed with Dowidar and Khalifa [28]who detected *E. coli* strains 40% in raw milk While, higher results were obtained by Kandil et al., [29]who detected *E. coli* was observed in 60%of the examined samples. While Rahman et al.,[30] detected *E. coli* with a percentage of 29.63%.

The high incidence of *E. coli* isolation from milk may be attributed to fecal contamination of the udder. Presence of *E. coli* in milk not only regarded as an indicator of fecal contamination, but more likely as an indicator of poor hygiene and sanitary practices during milking and further handling.[31]

Also, the isolation of *E.coli* in cheese was (40%) which nearly agreed with Abdeltawab et al., [32]the eventual outcomes incidence of *E. coli* in the analyzed samples were 42%.While, higher results were obtained by Imre et al., [33] who detected *E. coli* in 83.8% of the examined samples. On the opposite side, Stephan et al., cheese samples obtained in the year 2006 and year 2007, (3.7%) and (6.3%)[34]. Variable reported incidence rates could be influenced by many factors such as different techniques of cheese production, storage conditions, Cheese made from raw or pasteurised milk, as well as workers' personal hygiene and unsanitary production conditions.

The isolation of *E.coli* in yogurt was (8%) agreed with Ibrahim et al. who detected *E. coli* (8%) in yoghurt samples[35]. While higher results were obtained by Fahim et al. Detected by who detected *E. coli* in 30%, of the examined samples[36]. The variation among the results may be due to differences in manufacturing practices, climate, variation in sampling location, handling from producers to consumers and storage.

E. coli isolates in this study were subjected to the different biochemical tests. The results revealed Oxidase negative, TSI (A/A/Gas) negative, Urease test negative, Vogous Proskauer negative, citrate negative as well as positive reaction for indole and methyl red. The similar biochemical identification of *E.coli* through the traditional biochemical tests was previously described before in several studies such as Raji et al.,[37] , Surendraraj et al.,[38]; Abd El- Tawab et al.,[39]

E.coli isolates are serologically divided into serogroups on basis of their antigenic composition (somatic O antigens) [31]. Ten *E.coli* isolates were examined serologically and the most predominant serogroups were O44:K74 and O78:K80 (30%, each) followed by O114:K90 , O125:H21 and O111:H2: k58(20%, each) similar to Sobeih et al. [40]who detected O44:K74 and O78:K80 from the examined samples. It is also similar to Megawer et al., who detected O125:H21[41], Rizq et al., who detected O114:K90 [7], and Bagoury who detected O111:H2 [42]. Younis et al., Isolated *E. coli* demonstrated that 10 out of 100 milk samples were contaminated with *E. coli*. Depending on serology, the isolates were classified as O114, O111 , O125.[43]. Similar to this study, the results obtained by Sobeah et al.,[40] examined samples were similar , as pathogenic serotypes were identified : O78:K80, O44:K74, O114:K90, , O125: H21 and O111: H2 .

Regarding the antimicrobial sensitivity results for 10 *E.coli* strains in the current study, all isolates exhibited high sensitivity to gentamicin (90%), linezolid (90%), kanamycin (80%), and streptomycin (60%) while resistance was recorded for penicillin, trimethoprim-Sulfamethoxazole (Cot25), chloramphenicol (100%), cotrimoxazole 25 (90%), ampicillin (70%), cefotaxime (70%).

These findings were similar to Igbinosa et al.[44] who reported higher resistance of *E. coli* strains for penicillin and ampicillin (100%), chloramphenicol (94.7%). sulfamethoxazole(78.9%), while were sensitive to gentamicin(100%) and kanamycin (97.6%) and near near to Gundogan and Avci study [45], which stated that there is a high resistance of *E. coli* to ampicillin (90.5%), penicillin (82.1%), trimethoprim/sulfamethoxazole (44.2%), chloramphenicol (29.4%)[45]. The finding was similar to Jhandai et al., [46] the *E. coli* isolates from raw milk were highly resistant to penicillin (95.65%), streptomycin (56.52%), and cefotaxime (76.92%). Also, Diaz et al., [47] *E. coli* isolates were 100% resistant to penicillin and chloramphenicol. While, Tadesse et al., [48] isolated highly resistant *E. coli* to ampicillin (70%), sulfamethoxazole-trimethoprim

(60%),and chloramphenicol (50%). The results was also different from Beier et al.[49], *E. coli* was found Resistance to sulfamethoxazole-trimethoprim (10.5 %), streptomycin (7 %), and chloramphenicol (7 %) . Variations in the percentages of antimicrobial susceptibility may be attributed to the differences in the concentrations of antibiotic agents, differences in the geographical location, locally approved drugs, farm-level management and misuse or overuse of antibiotics.[50]

Gamma radiation is an effective method for reducing or eliminating E. coli. It damages the bacteria's DNA, by disrupting the genetic code and interfering with replication and protein synthesis, leading to cell death or preventing replication. [51] It also causes forming of free radicals which damage bacterial cells.[52] The efficiency of gamma irradiation on E. coli depends on the irradiation dose[22]. In this study, *E. coli* showed reduced activity at 2 and 4 kGy which is similar to Larasati et al.[51] which indicated that The dose needed to eliminating *E. coli* is., (≥ 3 kGy).and similar to Deng et l., [53]which showed that irradiation at doses 4.00 kGy was appropriate for eliminating almost all *E. coli* .

The numbers of inoculated E. coli decreased gradually with increasing irradiation doses. these results is similar to Ebraheem et al.[54] that stated that irradiation of the inoculated samples at doses 4 and 6 kGy significantly decreased the counts of the inoculated pathogen compared with the control. Gamma irradiation can also trigger *E. coli* stress responses, including activation of DNA repair pathways, changes in metabolism, and alterations in virulence mechanisms. Studies have shown that irradiation can alter the expression of genes related to antibiotic resistance, affecting how the bacteria responds to antibiotics.[52]

Gamma irradiation can affect the antibiotic susceptibility of *E. coli*, by increasing or decreasing it, depending on the irradiation dose and the specific antibiotic. Sub-lethal doses can alter the organism's response to certain antibiotics, while higher doses can lead to a reduction in antibiotic resistance[55]. In this study, there was altered antibiotic susceptibility for some samples. Decreased resistance for some antibiotics was observed and changed from resistant to sensitive in some samples with increase in irradiation doses. That could mean altered or changed gene responsible for *E. coli* resistance for these antibiotics. We selected an *E. coli* isolate that gamma irradiation changed its antibiotic susceptibility for chloramphenicol and co-Trimoxazole from resistant to sensitive, showed best growth according to total *E. coli* count and biomass through different gamma irradiation doses. The selected isolate was examined for detection of resistance genes *(flo*R gene *and dfr*A gene) before and after gamma irradiation by PCR.

The development of PCR-based methods provides a promising option for the rapid identification of bacteria and genes. With this method, identification of bacterial pathogens can be made in hours, rather than days, as conventional cultural methods require. PCR can also improve the level of detection due to its high sensitivity. Theoretically, only a few cells of pathogen are necessary to yield a positive diagnosis [56]. The floR gene is a bacterial gene that contributes to resistance to the antibiotic florfenicol and chloramphenicol. It is often found on plasmids or chromosomes[57, 58]. The floR gene encodes a protein that acts as an efflux pump, actively transporting the antibiotic out of the bacterial cell, thus reducing the drug's effectiveness inducing resistance[59]. The dfrA gene is a type of bacterial gene that contributes to resistance to the antibiotic trimethoprim by encoding a Dihydrofolate reductase (DHFR) enzyme that is insensitive to the drug. DHFR is an enzyme crucial for bacterial folate metabolism, which is essential for DNA synthesis. Trimethoprim inhibits DHFR, thus blocking bacterial growth. The dfrA gene encodes a modified version of DHFR that is not inhibited by trimethoprim. This allows bacteria carrying dfrA to survive and multiply even in the presence of the antibiotic[60].

*flo*R gene -responsible for chloramphenicol resistance- PCR test was positive in a pathogenic isolate of *E. coli* of this study before irradiation, this similar to Wang et al., [61]. *E. coli* was characterized molecularly by virulence determinants for *flo*R gene by PCR analysis after gamma irradiation and the results were

negative. This explains decreased antibiotic resistant of this isolate for chloramphenicol. Also, *dfr*A gene -responsible for trimethoprim resistance- PCR test was positive in a pathogenic isolate of *E. coli* before irradiation. The *dfr*A gene was detected similar to Rešková et al., [62]. After gamma irradiation the PCR test for *dfr*A gene was negative, which explains decreased antibiotic resistant of this isolate for trimethoprim.

Gamma irradiation decreased antibiotic resistance for some antibiotics in some samples, this is similar to Merdash et al.,[63] study which showed increased susceptibility of *E. coli* to colistin and quinolones antibiotics after gamma irradiation due to reduction in the expression levels of both *mcr*-1 and *qnr*-S genes. In addition, *E. coli* inhibited the growth of *Klebsiella* after exposure to 4 kGy of gamma irradiation. Which means changed virulence factors of the *E. coli*, altered protein synthesizing, lead to production of antimicrobial compound such as colicins that has an antimicrobial effect on other organisms. According to Kwon et al., [64] these virulence factors enable *E. coli* to live extraintestinal life and proliferate. Sub-lethal doses of gamma irradiation have a potential effect on *E. coli*

5. Conclusions

A total of 32 isolates of multidrug resistance *E. coli* were recovered from 100 samples representing raw milk, cheese and yogurt. MDR *E. coli* isolates containing dfrA and floR resistance genes reverted their sensitivity and switched off both genes after exposure to gamma irradiation at doses of 2 and 4 KGy. Gamma Irradiation was found to have a significant effect that led to decreased total E. coli count, bacterial cell mass. So, we can conclude that gamma irradiation is an efficient method to eliminate antimicrobial resistance genes from *E. coli* contaminating milk and dairy products.

Authors' declarations

Publication consent

Each author has demonstrated their consent for the publication of the current manuscript.

Data and material availability:

All data of this study is provided.

Conflict of interests.

All authors have stated the absence of any conflicts of interest.

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