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The feasibility of gamma radiation in reducing *Bacillus cereus* in meat and poultry products

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ABSTRACT

he purpose of the conducted study was to verify the existence and pathogenicity of Bacillus cereus (B. cereus) in meat and poultry products by evaluating the effect of gamma radiation on contaminated chicken fillets meat. A total of 100 samples of meat and poultry products represented by minced meat; sausage, rice kofta, chicken fillet and nuggets (20 of each) were collected randomly from different markets in Zagazig, Sharkia Governorate, Egypt, and subjected to sensory and bacteriological examination. The results that 20% represented the total prevalence rate of B. cereus in all examined samples, which were tested for the presence of four virulence genes: hbl, cytK, nhe and ces. All isolates exhibited traits related to virulence. The most predominated gene was cytK and ces (57.1%) for each, followed by nhe and hbl genes (42.8% and 28.5%), respectively. Experimental protocol for several treatments showed that Gamma radiation has no significant effect on the sensory characteristics of the examined chicken fillet sample, on the other side gamma irradiation at a dose of 1 kGy and 3 kGy reduce initial count from 5.83 log CFU g-1, to 3.31 log CFU g-1 and 2.04 log CFU g-1 at 1 kGy and 3 kGy respectively, while, for complete suppressing of B. cereus, the Gamma irradiation needed was 5 kGy where the viable cells was not detected in the tested samples.

INTRODUCTION

Bacillus cereus, a Gram-positive, rod shaped endospore-forming bacteria is considered a relatively common cause of gastroenteritis worldwide without any particular distribu-

tion. Food intoxication caused by *B. cereus* includes two types of gastrointestinal illness via elaboration of one emetic toxin and three different enterotoxins: an emetic (vomiting) syndrome or a diarrheal syndrome (**Per and**

Corresponding Author: Huda kamal Hussien E-mail: dr.hudakamal@gmail.com DOI: 10.21608/ejah.2023.320656 **Terje 2006).** While the emetic form occurs due to ingestion of food contains emetic toxin (cereulide), the diarrheal syndrome occurs after ingestion of food contaminated with *B. cereus* itself that secretes its enterotoxins in the intestine. The infective dose of *B. cereus* in emetic food poisoning is 10^4 to 10^{11} cfu/g or ml, while in diarrheal food poisoning; it is between 10^7 and 10^{10} cfu/g (**Granum 1997**).

As a ubiquitous microorganism *B*. cereus commonly found in the soil and able to producespores resistant to heat and desiccation, B. cereus is not uncommon to be isolated from wide variety of both raw and cooked (RTE) foods. Presence of spores enables B.cereus to survive stress conditions, so it is highly found in dry grains like rice, otherrice-based products and foods, such as pasta and noodle. Spores can survive perfectly in the dehydrated rice, without loss of viability for at least 48 weeks of storage (Rodrigo et al. 2021). Moreover, it easily spreads to the foods of plant origin and through cross-contamination to other foods such as milk, meat and meat products. (Tewari et al. 2015).

Therefore, it was required to find a proper control method for microorganism and its enterotoxins production and at the same time doesn't alter the sensory properties of the products. The current trends in consumers' demands that ask for additive free, fresher and more natural food products while maintaining microbiological safety (Gould 1996). These needs could be accomplished by ionizing radiation, which has been shown to inactivate food borne pathogen and spoilage microorganisms with a minimal alteration of food taste, flavor or nutritive value (Patterson et al. 2000).

Food irradiation is a process that has proven to be successful, not only in keeping the safety, but also in extending the shelf life of fresh meat due to its high effectiveness in inactivating pathogens without deteriorating product quality (Mahapatra et al. 2005). Ionizing radiation in general is produced by Gamma radiators and can kill microorganisms without rising the temperature of irradiated material. Irradiation damages microbial cell components, including DNA and the cytoplasmic membrane. Irradiation sensitivity of foodborne microorganisms is affected by intrinsic and extrinsic factors. Microorganisms that exhibit increased radiation resistance seem to have efficient mechanisms for repairing damaged DNA (Mendonça and Daraba 2014).

The purpose of the existing study was intended to determine the incidence of *B. cereus* in different types of meat and poultry products such as minced meat, sausage, rice kofta, chicken fillet and chicken nuggets as well as studying the effect of gamma irradiation on *B. cereus*.

MATERIAL and METHODS

One hundred random samples of meat and poultry products represented by minced meat; sausage, rice kofta, chicken fillet and nuggets (20 of each) were collected from different markets in Sharkia governorate, Egypt. The samples werereserved in a separated sterile plastic bag, labeled and conserved in an ice box then transferred to the laboratory. The samples were prepared according to ISO 6887- 3:2017.

Detection and eumeation of B. cereus according to FDA, BAM (2012), chapter-14, rev. 2020: Inoculate duplicate (Mannitol Egg Yolk Polymixin – MYP) Agar plates with each dilution of sample (including 1:10) by spreading 0.1 mL evenly onto surface of each plate with sterile glass spreading rod. Incubate plates 18-24 h at 30°C and observe for colonies surrounded by precipitate zone, which indicates that lecithinase is produced. B. cereus colonies are usually pink color on MYP. Pick up 5 or more pink, lecithinase- positive colonies and transfer to nutrient agar slants for furtherconfirmation (Phenol red glucose broth, Nitrate broth) Modified VP medium, Tyrosine agar and Lysozyme broth). Isolated strains of B. cereus appear as large Gram- positive rods with spores that do not swell the sporangium; produce lecithinase and do not ferment mannitol on MYP agar; grow and produce acid from glucose anaerobically; reduce nitrate to nitrite (a few strains may be negative); produce acetylmethylcarbinol (VP-positive); decompose L-tyrosine; and grow in the presence of 0.001% lysozyme.

Polymerase Chain Reaction (PCR): -

2.1. DNA extraction. DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations.

2.2. Oligonucleotide Primer. Primers used were supplied from Metabion (Germany) are listed in table (A).

2.3.- PCR amplification.

PCR, primers were utilized in a 25- μ l reaction containing 12.5 μ l of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 μ l of each primer of 20 pmol concentration, 5.5 μ l of water, and 5 μ l of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

2.4.- Analysis of the PCR Products.

The products of PCR were separated by electrophoresis on 1% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 μ l of the products was loaded in each gel slot. Generuler 100 bp ladder (Fermentas, Germany) and gelpilot 100 bp plus ladder (Qiagen, gmbh, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table A. Primers sequences, target genes, amplicon sizes and cyclingconditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturat	Amplification (35 cycles)		Final extension	Reference Ehling- Sc	
			ion	Secondary denaturatin	Annealing	gExtension		hulz et al. 2006
hbl	GTA AAT TAI GAT	1091	94°C	94°C	49°C	72°C	72°C	
	GAI CAA TTTC AGA ATA GGC ATT		5 min.	30 sec.	40 sec.	1 min.	10 min.	
	CAT AGA TT							
nh	AAG CIG CTC TTC	766	94°C	94°C	49°C	72°C	72°C	
е	GIA TTC		5 min.	30 sec.	40 sec.	45 sec.	10 min.	
	ITI GTT GAA ATA							
	AGC TGT GG							
cyt	ACA GAT ATC GGI	421	94°C	94°C	49°C	72°C	72°C	
K	CAA AAT GC		5 min.	30 sec.	40 sec.	45 sec.	10 min.	
	CAA GTI ACT TGA							
	CCI GTT GC							
ces	GGTGACACATTAT CA	1271	94°C	94°C	49°C	72°C	72°C	
	TATAAGGTG GTAAGCGAACCT		5 min.	30 sec.	40 sec.	1.2 min.	10 min.	
	GTC							
	TGTAACAACA							

Decontamination by gamma irradiation:

The irradiation process was carried out at the National Center for Radiation Research and Technology (NCRRT) At Nasr City, Cairo, Egypt. The irradiation facility used was Indian Gamma Cell (Ge 4000A), dose rate 2.2 KGy/ hour. The radiation source was Cobalt60 which assure uniform gamma irradiation of the experimental samples. The chicken fillets samples were divided into four groups; the control group and three treated groups subjected to 1, 3 and 5 kGy (Moini et al. 2009) (each group contained five fillet samples). The tests were done 5 trials. Upon completing the desired passes, each package was returned to ice box and transported to microbiology laboratory for analysis and enumeration of B. cereus, after sensory evaluation according to Wierbicki (1985) where the coded examined samples were organoleptically examined for color, odor and texture using untrained panel consisting of 10 members. The panelists used a 9-rating scale for their scores (Scores above 5 indicated acceptable products, scores 5 and 4 indicated that the product of marginal quality, whereas scores of 3 and below indicated that product might not be accepted by the consumer.

4-Statistical analysis

The obtained data were statistically analyzed using analysis of variance (ANOVA) test and comparative of means were performed according to Duncan, Multiple Range test for comparison of Means using SPSS ver. 14 (2006). All microbial counts were converted to the base – 10 logarithm of the number of colony forming units per g of examined samples (log CFU/g). Results were recorded as mean \pm standard errors (SE) also minimum (Min) and maximum (Max) were calculated. The value of **P** < 0.05 was used to indicate statistical significance.

RESULTS

The prevalence of *B. cereus* in various food products was illustrated in Table (1). Among total of 100 samples, *B. cereus* was isolated by 20%, the most frequent prevalence was recorded in Rice kofta and sausage with 30 % and 25 % respectively, followed by minced meat, sausage, pane and nuggets (15%) for each. All the isolates were motile and hemolytic on 5 % sheep blood agar.

Bacillus cereus count as shown in table (1) ranged from 3.77 to 2.68 log CFU g-1.While the highest contamination rate was found in Rice Kofta and nuggets samples (3.77 log CFU g-1 for each), the lowest rate was recorded in pane samples. While, minced meat and sausage recorded (2.92) and (3.13) log CFU, respectively.

The isolates were screened by multiplex PCR for the presence of four enterotoxin genes as showed in table (2) and photos (1-4), having the predicted size: 1091, 422, 766 and 1271 for *hbl*, cytK, *nhe* and *ces*, respectively.

The prevalence of the emetic gene *ces* among all *B. cereus* isolates in the current study was57.1%. The *ces* gene was only detected in isolates from Rice kofta (28.5% of isolates), pane(14.2) and nuggets (14.2%), while from the tested 20 isolates, (28.5%), (57.1%) and (42.8%) were able to produce the *hbl*, *cytK* and *nhe* virulence genes respectively, which may have the ability to synthesis of functional gene products expressed as enterotoxins.

As shown in table (3) the effect of gamma irradiation on organoleptic characters of chicken fillets with no significant difference in the scores given by the judges for the color, odor and texture of the examined samples.

Turning to data illustrated in table (4) and figure (1) It was observed that up to 3kGy of γ -irradiationdose was not enough to eliminate all vegetative (2.04 log CFU/g) *B. cereus* cells in chicken fillet samples, the total elimination was achieved at 5 kGy.

Type of samples		Positive	Mean count \pm SE	
	-	No.	%	
Meat	Minced meat	3	15	2.92 ± 0.42
products	Sausage	5	25	3.13 ± 0.27
	Rice kofta	6	30	3.77 ± 0.21
Chicken products	Pane	3	15	2.68 ± 0.45
	Nuggets	3	15	3.77 ± 0.39

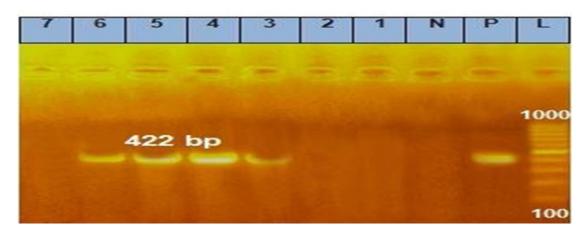
Table 1. incidence ratio and count of *B. cereus* in meat and poultry products

Table 2. PCR screening of *B. cereus* virulence genes

Serial No.	<i>B. ceries</i> isolates	hbl	cytK	nhe	ces
1	Minced meat	-	-	-	-
2	Sausage	+	-	-	-
3	Rice kofta	+	+	+	+
4	Rice kofta	-	+	+	+
5	Sausage	-	+	-	-
6	Pane	-	+	-	+
7	Nuggets	-	-	+	+



Photo(1): Agarose gel electrophoresis of multiplex PCR of hbl gene (1091bp) for characterization of B. cereus



Photo(2): Agarose gel electrophoresis of multiplex PCR of *CytK* gene (422bp) for characterization of *B. cereus*

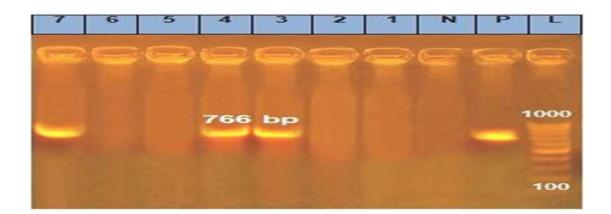


Photo (3): Agarose gel electrophoresis of multiplex PCR of *nhe* gene (766bp) for characterization of *B*. *cereus*



Photo (4): Agarose gel electrophoresis of multiplex PCR of *ces* gene (1271bp) for characterization of *B. cereus*

Table 3. Effect of gamma irradiation on organoleptic properties of examined chicken fillet samples

Dose of gamma irradiation	Non treated	1KGy	3KGy	5KGy
General appearance	7.7 <u>+</u> 0.3	7.5 <u>+</u> 0.3	7.3 <u>+</u> 0.21	7.4 <u>+</u> 0.27
Odor	7.8 <u>+</u> 0.32	7.7 <u>+</u> 0.3	7.4 <u>+</u> 0.26	7.1 <u>+</u> 0.2
Texture	7.9 <u>+</u> 0.31	8 <u>+</u> 0.29	7.8 <u>+</u> 0.36	7.1 <u>+</u> 0.27

Mean values for rating scales according to Wierbicki (1985)

Means within the same row carrying different superscripts are sig. different P< 0.05 based

Dose sample	Initial count	1KGy	3KGy	5KGy
Chicken fillet samples	5.83± 0.21 ^a	$3.31{\pm}0.14^{\text{b}}$	$2.04\pm\!0.07^{c}$	ND
Log reduction		2.52	3.79	
D ₁₀ value	<u>_</u> _	0.40	0.79	

Table 4. Effect of gamma irradiation on *B. cereus* count (\log_{10} CFU/g) of chicken fillet samples

Significance differences (P < 0.05) High Significance differences (P \le 0.01) according to the result of stat. ND: < 1 log₁₀

Log reduction = $\log_{10}(X_0-X) D_{10}$ = Radiation dose/ $\log_{10}(X_0-X)$

Where X_0 is the initial number of organisms, and X is the number of organisms surviving the radiation dose.

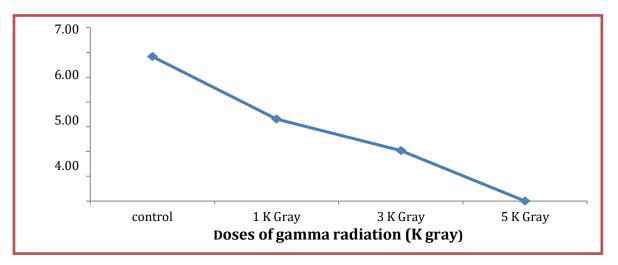


Figure (1) Effect of gamma irradiation on *B. cereeus* count (\log_{10} CFU/g) of chicken fillet samples

DISCUSSION

As mentioned in table (1), B. cereus was isolated in this study from 20 of 100 different food samples screened making an overall percent incidence of 20%, almost similar incidence was reported by Schlegelova et al. (2003) who found 28 % of meat samples positive for B. cereus and Willayat et al. (2007) who recorded 23.5 % of samples were positive, while higher results were reported by Tewari (2013) and Das et al. (2009) who found 30.6 % and 36.7 % contamination level of B. cereus, respectively. This variation is fairly acceptable and it may be due to samples variation and different hygienic practices followed in meat shops and restaurants in different localities.

Rice Kofta samples which represented the highest prevalence throughout \mathbf{d} tested samples, the results were lower than those recorded by Edris et al. (2005) and Hassan et al. (2019)) % • and 56.7%), respectively.

The incidence of *B. cereus* in sausage samples was 25%, this result came in line with **tw** reported previously in Egypt by **Tharwat et al. 2020 (32%) and Eid-Amal et al. 2008** (30%), however, higher results were reported by **Edris et al. (2005) and Hassan et al. (2019)** who recorded 40% and 46.7%, respectively. While, minced meat, nuggets and pane samples recorded 15% for each. The results were lower than that previously reported in Egypt by Tharwat et al 2020 (76%) in minced meat, while it was found to be 44% and 28% for pane and

nuggets respectively, according to Abd El Tawab et al. (2020). In the present study, B. cereus prevalence was the highest in Rice Kofta which may bedue to its composition and chemical characteristics which serve as excellent medium for B. cereus at different temperatures. Moreover, B. cereus spores can survive perfectly in the dehydrated rice, without loss of viability for at least 48 weeks of storage and easily revigitate when there are suitable conditions (Jaquette and Beuchat 1998). On the other hand, cross-contamination, some defects in the hygienic measures, or the type of additives or spices used in sausage production can explain the high prevalence rate of B. cereus in the examined sausage samples.

Overall counts of *B. cereus* in all examined samples were ranged from 3.77 to 2.68 log CFU g-1. In support of this, **Berthold-Pluta et al. 2019** reported similar levels of *B. cereus* count (from 3 to 2.1log CFU g-1), as well as **Tewari et al. (2015** (2.91 to 4.1log CFU g-1).

Optimistically, results in the present study are within acceptable limits for consumption as the value quoted for the minimum infective dose for the diarrheal illness is generally higher than 10⁷ cfu/gram (Granum and Baird-Parker 2000) and for emetic illness, where toxin is preformed in the food, requires a higher cell concentration (10^7 to 10^8 CFU/g) to produce clinically significant amounts of toxin (Tajkarimi 2007). Despite that, there are reported cases of both emetic and diarrheal diseases involving lower levels (below 10^7 cfu/g) of *B. cereus*. EFSA (2016) remarkably, the highest prevalence rate and count of B. cereus in present study were recorded in rice kofta samples, this could be understandable as B. cereus has been frequently associated with foodstuffs that contain rice; however other food products, such as pasta and noodles could be also a vehicle for contamination and being involved in B. cereus intoxication (Grande et al. 2005). Virulence genes hbl, nhe and cytotoxiny K are primarily responsible for producing diarrheal enterotoxins form of B. cereus, while ces gene is the one that responsible for the emetic form of *B. cereus* intoxication as illustrated in table (2).

Considering the enterotoxigenic profile of isolates in this study, the most prevalent toxin gene was *cytK* represented by 57.1%. This came in line with **Owusu-Kwarteng et al** (2017) who reported *cytK* gene as the dominant gene throughout the tested isolates (75%) and **Mostafa et al.** (2022) who detected 61.11% isolates carrying *cytK* gene. Following *cytK* gene, were *nhe and hbl* genes with 42.8% and 28.5%, respectively. Similar results were obtained by (Rahimi et al. 2013) who recorded 44.04% and 34.52%, while higher results 80% and 70% for *nhe* and *hbl*, respectively. Were reported by (Abd El Tawab et al. 2020)

The prevalence rate of the emetic toxin gene *ces* was 57.1%. Significantly lower rates (1.5 to 17.2%) were obtained from previous studies (Owusu-Kwatreng et al 2017; Yim et al 2015; Hoton et al. 2009). The difference in rates being attributed to the variations in food property as mentioned by Zuberovic et al. (2014).

Regarding to the sensory effect of gamma irradiation on chicken meat, it was observed that no significant effect on color, odor or texture. This may be due to using low radiation doses, similar result recorded by Abu-Tarboush et al. (1997) who observed that irradiation doses (2.5, 5.0, 5.7 and 10.0 kGy) of chicken meat caused minor changes in product acceptance in relation to appearance, aroma, texture and flavor. Mantilla et al. (2012) found that all the irradiated fillets showed a color more attractive than non-irradiated appearance of such attractive color was attributed to Comioglobina caused by the interaction with carbon monoxide during irradiation Nam and Ahn (2002).

Determining the irradiation effect on *B*. *cereus* count `in chicken fillet was illustrated in table (3). Viable cell count was recorded before and after exposure to Gamma irradiation. The initial count was $5.83 \log \text{CFU g-1}$, that reached $3.31 \log \text{CFU g-1}$ and $2.04 \log \text{CFU g-1}$ when exposed to 1 kGy and 3 kGy respectively, while for complete suppressing of *B. cereus* the Gamma irradiation needed was 5 kGy where the viable cells was not detected in the tested samples.

The calculated D10 value was 0.4 at 1kGy but this value increased progressively after exposure to 3 kGy. Nearly similar results recorded by Ayari et al (2012)who said that D10 was 0.42 at 1kGy and the value increased to 0.49 after the fourthcycle at the same radiation dose (1 kGy).

As noticed from the line chart in figure (1) the decrease in *B. cerues* count was rapid at the beginning till 1 KGy with 2.52 log reduction after that the reduction in counts increased at 3 kGy with 3.79 log reduction. This may be explained as the very first doses of radiation eliminated the vegetative cells, so the reduction of the count was rapid. (Abo-State 1991 and 1996).

CONCLUSION:

he higher prevalence rate of *B*. cereus group isolates in meat products such as rice kofta and sausage recorded in the present study, giving indication about the habitat of the tested microorganismand the possibility of cross contamination or through the contaminated additives. Side by side, virulence genes responsible for both diarrheal and emetic illnesses were obtained in recent study, highlighting the public health and economic value of *B. cereus* and the urgent need of applying strict hygienic measures during meat processing. But on the bright side, complete eradication of B. cereus at 5 kGy Gammaradiation, creating safe and time saving control method for B. cereus in food, and at the same time, it does not negatively affect the sensory properties of the product, which are of interest to the consumer.

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