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Incidence and Radiation Sensitivity of *Bacillus cereus*, *Listeria monocytogenes* and Their Toxins in Some Chicken Products

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Abstract: Thirty samples of chicken luncheon and minced chicken (15 samples for each) were collected from Cairo and Giza supermarkets. The mean values of total aerobic plate count (APC), *Bacillus* spp. and *Listeria* spp. recovered from chicken luncheon were 1.33×10^7, 7.53×10^4 and 6.67 CFU/g, respectively. However these values for minced chicken were 5×10^6, 1.4×10^4 and <100 CFU/g, respectively. The incidence of *Bacillus cereus* in chicken luncheon was 53.3% and in minced chicken was 60%. *Listeria monocytogenes* was not isolated in chicken luncheon and in minced chichen the microorganisms was found in 6.7%, of the samples. Toxicity test revealed that two out of 8 and one out of 9 isolates of *Bacillus cereus* .from chicken luncheon and minced chicken, respectively and one strain of *Listeria monocytogenes* isolate from minced chicken presented mice lethal toxin. The D_{10} value of the two highly toxigenic strains of *Bacillus cereus* and *Listeria monocytogenes* after exposure to gamma irradiation was 1.9 and 0.4 kGy, respectively. Gamma irradiation had lethality effect on *Bacillus cereus* and *Listeria monocytogenes* toxins when 20 kGy was used. Ten kGy gamma radiation activated both toxins lethality.

Key words: *Bacillus cereus* · *Listeria monocytogenes* · Toxins · Chicken products · Gamma radiation · Dose response curve

INTRODUCTION

Bacillus cereus is a sporeforming, Gram positive, aerobic, rods bacteria. It has been long known as ubiquitous organism found in air, soil and water [1]. *Bacillus cereus* is the aetiologic agent of two distinct types of food poisining characterized either by diarrhea and abdominal pain or by nausea and vomiting after ingestion of contaminated foods [2].

Listeria monocytogenes is an emerging food borne Gram positive non spore forming bacilli. It is commonly found in soil, water and decaying plant material. *Listeria monocytogenes* can survive and grow over a wide range of environmental conditions as refrigeration temperatures, low pH and high salt concentration. This allows the pathogen to overcome food preservation and safety barriers and pose a potential risk to human health. It is a frequent post process contaminant of ready to eat meat products. *Listeria monocytogenes* is the causative agent of Listeriosis, a severe disease with high hospitalization and case fatality rates. The disease has a long incubation time, which makes it difficult to identify the pathogen and trace the contaminated food [3].

Microorganisms control in meat products is the major concern in the preparation of high quality foods. During slaughtering process the meat is exposed to many sources of contamination [4]. The hygienic state of animals prior, during and after slaughter can be critical to the finished product quality [5].

Irradiation is known to be the best method for the control of potentially pathogenic microorganisms in meat without affecting its physical state [6]. Food irradiation is generally defined as the process in which foods are exposed to certain forms of ionizing energy from radioactive sources mainly gamma rays. Cobalt-60 is a

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highly penetrating source of ionizing radiation used in food either fresh or after processing and packaging. Food safety officials and scientists view irradiation as an effective point in Hazard Analysis and Critical Control Points (HACCP) established for meat and poultry processing because of its effectiveness in minimizing the possibility of cross-contamination prior to consumer use [5].

The US Center for Disease Control (CDC) estimated that if half of the ground beef, pork, poultry and processed luncheon meats in the US were irradiated, there would be over 880,000 fewer cases of food borne illness, 8500 fewer hospitalizations, 6660 fewer catastrophic illnesses and 352 lives saved every year [7].

The radiation resistance of a specific organism may vary according to the environment in which it is irrradiated [8]. The D_{10} values (dose of ionizing radiation required to eliminate 90% of the microorganism) of bacteria in food are affected by a number of factors, such as water activity, composition, irradiation temperature, presence of oxygen. In addition, some of the constituents of complex food system, such as proteins, are thought to compete with the cells for the interaction with radiolytic free radicals, thereby reducing the net effect of radiation damage and making the organisms sometimes more radiation resistant [9].

The objective of this study was to evaluate the microbial load (total aerobic plate count, *Bacillus* spp. and *Listeria* spp.), the incidence of *Bacillus cereus, Listeria monocytogenes* in some chicken products and the effect of gamma rays on the dose response curve of both pathogens and their toxins.

MATERIALS AND METHODS

Sampling: Thirty samples of chicken luncheon and minced chicken (15 samples each) were bought from different supermarkets in Cairo and Giza, to determine their microbial load (total aerobic plate counts, *Bacillus* spp. and *Listeria* spp.) and detect the presence of *Bacillus cereus* and *Listeria monocytogenes.*

Microbiological Analysis: Twenty five grams of each sample were homogenized in 225 ml sterile physiological saline solution (0.85% Nacl) using a Stomacher model 400 (Seward laboratory London) for 1-2 minutes, then decimal dilutions were prepared. Total aerobic plate counts were enumerated on Plate Count Agar (PCA) medium [10] using pour plate technique. *Bacillus* spp. were counted on Luria broth (L.B) medium and isolation of *Bacillus cereus* was carried out on Mossel's *Bacillus cereus* selective agar (MYP) medium [11] by spread technique on pre-poured plates which were identified, according to FDA [12]. *Listeria* spp. were counted on Palcam agar (CM877 plus SR 150 Oxoid) while *Listeria monocytogenes* isolation was done using UVM broth 1 (CM 863 plus 142 Oxoid) followed by UVM 2 (CM 863 plus 143 Oxoid) for enrichment and plated on Palcam agar [13] by spread technique. Suspected colonies were identified by Gram staining, catalase activity, motility at 25 and 37°C, β-haemolysis and carbohydrate fermentation [14].

Pathogenicity Test: Isolated strains of *Bacillus cereus* and *Listeria monocytogenes* together with standard *Bacillus cereus* (strain ATCC 11778) and standard *Listeria monocytogenes* (strain F 5069 serotype 4b) were used to prepare a suspension for *Bacillus cereus* [15] and for *Listeria monocytogenes* [14], respectively. Three mice (18-20g each) for each isolate were injected intravenous in the tail vein with 0.1 ml of the bacterial suspension. Three mice were kept as control and the death rates were reported.

Toxigenicity Test: The pathogenic strains of all previously mentioned strains were used to produce crude toxin according to Shinagawa *et al.* [16] for *Bacillus cereus* and Walton *et al.* [17] for *Listeria monocytogenes.* Each pathogen was inoculated on Brain Heart Infusion (BHI) agar slants and incubated at 37°C for 14-16 h. Then inoculate into 20 ml BHI broth in 100 ml conical flasks on a shaking water bath for 18 h at 37°C. For *Bacillus cereus*, one ml from the 16 h culture of each strain was inoculated into 100 ml BHI broth containing 1% glucose (BHIG) in 500 ml conical flasks for 16 h at 32°C with continuous shaking. Bacterial cells were removed by centrifugation at 8000 rpm for 20 min at 4 °C, followed by filtration of the supernatants through disposable millipore (0.45 μm) filters. The resulting filtrates were used as crude toxins. A volume of 0.5 ml of each crude toxin was injected intravenous in the tail vein of each mouse (18-20g). Three mice were used for each toxin and the death rates were reported.

Dose Response Curve: The D_{10} value was calculated for the most highly toxigenic strain of *Bacillus cereus* and *Listeria monocytogenes.* Thirty six samples of chicken luncheon and minced chicken were heat sealed in polyethylene bags (10g each). The samples were sterilized using 25 kGy by accelerated electrons (current, 2.1 m A

and speed 1.6 m/min) in National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. Sterility test was confirmed by aerobic plate counts. Isolated *Bacillus cereus* and *Listeria monocytogenes* strains were used for the artificial inoculation. One ml of the standardized inoculum was surface inoculated in each sterile sample and left overnight at 4°C. Inoculated samples were then exposed to 2, 4, 6, 8 and 10 kGy or 0.5, 1, 1.5, 2 and 2.5 kGy gamma irradiation doses for *Bacillus cereus* or *Listeria monocytogenes,* respectively, in NCRRT (3 samples for each dose), with a dose rate 4.3 kGy/h. The control samples (0.0 kGy) were left unirradiated. The log number of survivors was plotted against the absorbed radiation dose in kGy. Linear regression was applied using Excel program to produce the best fitting line for each treatment, from which the D_{10} values were calculated as the reciprocal of the absolute value of the regression line [18].

Effect of Gamma Rays on Toxin: A volume of 5 ml of crude toxin of the highly toxigenic strains (*Bacillus cereus* strain isolated from chicken luncheon, *Listeria monocytogenes* strain isolated from minced chicken and both standard strains) in screw capped test tubes were exposed to 10 or 20 kGy gamma irradiation and one tube from each toxin left unirradiated as control. Then 0.5 ml from each tube was injected in 3 mice and the death rates were reported.

RESULTS AND DISCUSSION

Results tabulated in Table 1 revealed the aerobic plate counts, *Bacillus* spp. and *Listeria* spp. counts contaminating 30 samples of chicken luncheon and minced chicken (15 samples each). The mean values were 1.33×10^7, 7.35×10^4 and 6.67 CFU/g for chicken luncheon samples, respectively and 5×10^6, 1.4×10^4 and < 100 CFU/g for minced chicken samples, respectively. *Listeria* species were undetectable (< 100) in all examined samples except one chicken luncheon sample (1×10^2 CFU/g) in which by identification it was other than *Listeria monocytogenes*. Zahran [19] previously failed to count *Listeria monocytogenes* in chicken luncheon. The total bacterial count is considered an index of quality which gives an idea about the hygienic measures during processing and helps in the determination of the keeping quality of the product [20]. High total aerobic plate counts might be attributed to the contamination of the product from different sources or unsatisfactory processing as well as it may be due to unsuitable condition during storage [21-22].

The incidence of *Bacillus cereus* (Table 2) in chicken luncheon and minced chicken was 53.3% and 60%, respectively, while *Listeria monocytogenes* was only 6.7% of the examined minced chicken samples. Although *Listeria* species were undetectable (< 100) in minced chicken but by isolation there were 5 positive samples

Table 1: Microbial load (CFU ml^{-1}) contaminating chicken luncheon and minced chicken

	Chicken luncheon			Minced chicken		
Sample	APC	*Bacillus* spp.	*Listeria* spp.	APC	*Bacillus* spp.	*Listeria* spp.
1	3.0×10^7	6.8×10^4	<100	1.5×10^6	2.4×10^4	<100
2	8.6×10^6	1.8×10^4	<100	8.5×10^6	3.2×10^4	<100
3	8.0×10^5	2.0×10^2	<100	9.6×10^6	8.1×10^4	<100
4	5.9×10^6	2.0×10^4	<100	7.8×10^6	3.1×10^4	<100
5	6.8×10^6	1.0×10^4	<100	1.0×10^7	2.5×10^4	<100
6	5.0×10^6	9.0×10^3	<100	4.0×10^7	6.0×10^5	<100
7	2.0×10^7	7.0×10^4	<100	8.3×10^6	3.9×10^4	<100
8	7.5×10^5	3.4×10^4	<100	1.8×10^7	5.0×10^4	<100
9	1.2×10^7	5.1×10^4	<100	8.5×10^6	2.8×10^4	<100
10	3.1×10^7	1.2×10^5	<100	1.0×10^7	4.0×10^4	<100
11	1.2×10^7	7.1×10^4	<100	8.9×10^6	1.2×10^4	<100
12	1.5×10^7	2.7×10^4	1.0×10^2	1.2×10^7	2.9×10^4	<100
13	1.3×10^6	3.0×10^4	<100	3.2×10^7	5.0×10^4	<100
14	8.0×10^6	1.0×10^3	<100	9.1×10^6	3.2×10^4	<100
15	2.0×10^7	1.1×10^5	<100	7.5×10^6	2.1×10^4	<100
Mean	1.33×10^7	7.53×10^4	6.67	5.0×10^6	1.4×10^4	<100

APC: Aerobic plate counts <100: undetectable

Table 2: Incidence of *Bacillus cereus* and *Listeria monocytogenes* in the examined samples

	Bacillus cereus		*Listeria monocytogenes*	
Samples	No	%	No	%
Chicken luncheon	8	53.3	0	0.0
Minced chicken	9	60.0	1	6.7

(not tabulated). By identification there was only one sample (6.7%) containing *Listeria monocytogenes* and the other 4 samples were other *Listeria* species. Hindy [23] isolated *Listeria* species from 28% and 20% of chicken fillet and meat samples, from which 8% and 4% were *Listeria monocytogenes*, respectively.

In a study done by Nortje *et al.* [24], the incidence of *Bacillus cereus* was higher in cooked and processed (ground beef) meat than in raw meat samples, also its presence at high levels, indicate a potential risk of producing toxins.

Bacillus cereus and *Listeria monocytogenes* are pathogens generally associated with red meat. Presence of *Listeria monocytogenes* might pose problems when considering its ability to proliferate at moderate to low temperature (with the risk of producing toxins) [25]. WHO considers that the primary mechanism of transmission of *Listeria monocytogenes* to humans is through food stuffs contaminated during production and/or storage [26].

Pathogenicity and Toxigenicity Test: The injection of the suspension of *Bacillus cereus* (8 and 9 isolates from chicken luncheon and minced chicken, respectively), *Listeria monocytogenes* (one isolate from minced chicken) and both standard strains revealed that all isolates were pathogenic resulting in the death of mice during the first day after injection (not tabulated). The toxigenicity of the tested strains indicated that four of them (3 *Bacillus cereus* strains and 1 *Listeria monocytogenes* strain) were lethal to mice. Comparing the lethality of the 3 isolated strains of *Bacillus cereus* with the standard *Bacillus cereus* ATCC 11778 and the isolated *Listeria monocytogenes* strain with the standard *Listeria monocytogenes* F 5069 serotype 4b revealed that only one strain *Bacillus cereus* from chicken luncheon and the only *Listeria monocytogenes* strain isolated from minced chicken were the greatest strains in toxicity. One of the three mice used for each strain died immediately after injection, another one died after one hour while the third one died overnight, meanwhile the three mice of the standard *Bacillus cereus* strain died overnight, while those of the standard *Listeria monocytogenes* strain did not die.

Abo-State [27] showed the powerful role of hemolysin in the toxicity and virulence of *Bacillus cereus* toxin(s). Wong *et al.* [28] found that 100% of 183 isolates gave positive hemolysin activity and 3 of 11 selected isolates showing strong haemolysin activity, killed adult mice. Garcia-Arribas *et al.* [29] found that 24 out of 39 *Bacillus cereus* strains gave positive mice lethal test. All tested strains possessed phospholipase activity.

The pathogenicity and toxigenicity, in mice of tested strains indicated the difference among the strains. This difference might be attributed to the difference in virulence of strains or to the immunity of the mice. Goepfert *et al.* [30] indicated that when the generalized infection dose occurred, it was possible that the host was in a weakened condition and unable to defend itself against the invading cells.

The Dose Response Curves: The dose response curves of survivors were done for the two highly toxigenic strains of *Bacillus cereus* isolated from chicken luncheon and *Listeria monocytogenes* isolated from minced chicken after exposure to different gamma irradiation doses (Fig. 1, 2). The D_{10} values for *Bacillus cereus* and *Listeria monocytogenes* was 1.9 kGy and 0.4 kGy, respectively. These results were in agreement with Abd El-Hady [31] who found that the D_{10} values of 3 strains of *Bacillus cereus* were 2.3, 2.2 and 2.0 kGy, while Abo-State [32] found that the D_{10} value was 1.5 kGy. On the other hand, the D_{10} value of *Bacillus cereus* in marinated beef ribs was 0.66±0.01 kGy [4], in roast beef meal components was 0.126-0.288 kGy [33], while in semi-dried seafood products was 0.64 kGy [34].

Patterson [35] investigated the sensitivity of *Listeria monocytogenes* on poultry meat finding that the D_{10} value was 0.42-0.55 kGy depending on the strain and plating medium used. Sommers *et al.* [36] obtained a D_{10} value of 0.6 kGy for *Listeria monocytogenes* surface inoculated onto frankfurters. At the same time, Sommers and Thayer [37] found it from 0.49 to 0.71 kGy with a mean value of 0.61 kGy. They speculated that product formulation and surface treatments were responsible for those differences. Conversely, *Listeria monocytogenes* has a D_{10} value of 0.2-2.0 kGy depending on the strain, substrate and culture conditions [38].

The importance of the D_{10} value is that it leads to a prediction of the dose required to inactivate any microorganism. Thayer *et al.* [39] reported that reduced

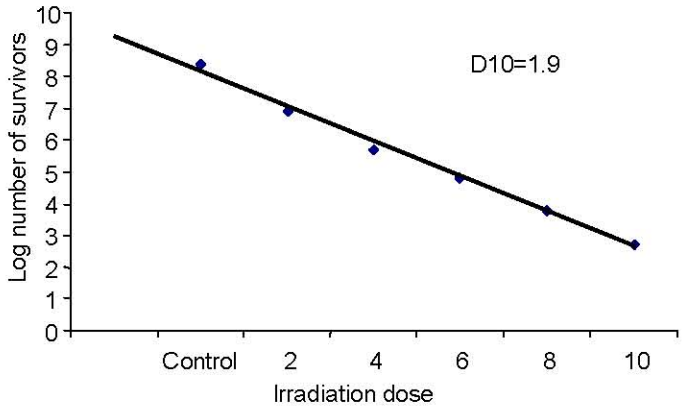

Fig. 1: Dose response curve of highly toxigenic *Bacillus cereus* isolated from chicken luncheon

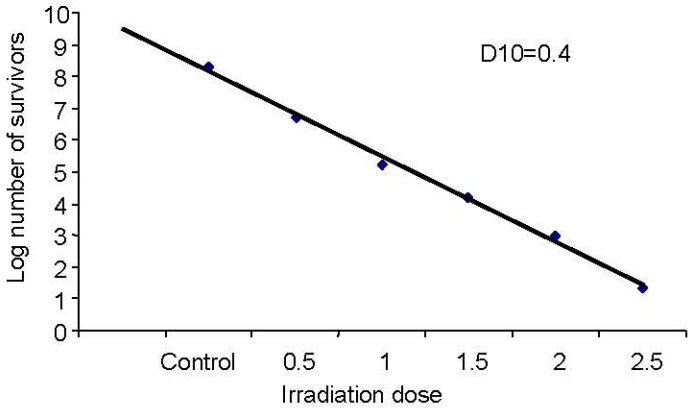

Fig. 2: Dose response curve of highly toxigenic *Listeria monocytogenes* isolated from minced chicken

water content or increased NaCl levels may result in the survival levels of the foodborne pathogens of irradiated meat to be greater than expected. Although [40] reported that gamma irradiation is not very effective against gram positive spore forming bacteria. Like *Bacillus cereus* and *Clostridium* spp. Shay *et al.* [41] required a 2-5 kGy dose of irradiation to destroy the vegetative bacteria of *Bacillus cereus*. A dose of 2 kGy gamma irradiation decreased growth and toxin production by *Bacillus cereus* in roast beef and gravy at abuse temperatures of 15 and 22°C [33]. While, Samelis *et al.* [38] suggested that 4 kGy may be sufficient to eliminate low levels (< 100 $cellg^{-1}$) of natural contamination with *Listeria monocytogenes* on meat.

Thayer and Boyd [2] stated that controlling stationary phase cells and endospores may be more important for food safety than controlling logarithmic phase cells because of the high radiation resistance of the endospore and because the stationary phase would probably be reached in most abused foods.

Effect of Gamma Rays on Toxins: After exposing the crude toxin of the highly toxigenic *Bacillus cereus* and *Listeria monocytogenes* strains to 10 kGy gamma radiation, two of the three mice used for each strain died immediately after injection, while the third one died within one hour. However the unirradiated control of each strain died within 16 hours. Meanwhile, the two crude toxins exposed to 20 kGy when injected in mice, one died immediately after injection, while the two others died within 14-16 hours. The results indicated that exposure to 10 kGy gamma irradiation did not affect the toxicity of toxins, *Bacillus cereus* toxin, but it activated its toxicity. Kamat *et al.* [42] reported a similar result that gamma irradiation had no effect on *Bacillus cereus* toxin lethality.

Finally, food irradiation can not be used to destroy microbial toxins nor viruses and spores at the low doses used to kill vegetative pathogens (below 10 kGy). It must be integrated as part of an overall good manufacturing practice program [5].

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