Effect of Gamma irradiation on viability of resistant bacteria *Salmonella enterica* serovar Kedougou contaminated in chicken meat and antimicrobial susceptibility of resistant bacteria

ผลของการฉายรังสีแกมมาต่อการมีชีวิตของเชื้อแบคทีเรียดื้อยาซา*ลโมเนลลา* ซีโรวาห์คิโดกุที่ปนเปื้อนในเนื้อไก่และความไวของสารต้านจุลชีพของแบคทีเรียดื้อยา

Nattharee Thanawan (นัฐรี ธนะวรรณ์)* Dr. Chanwit Tribuddharat (ดร. ชาญวิทย์ ตรีพุทธรัตน์)**

ABSTRACT

Many types of food can be contaminated with antimicrobial resistant bacteria (ARB). Gamma irradiation is one of the applications for food decontamination. The aim of this study was to determine the effect of Gamma rays on resistant bacteria in forms of bacterial suspension and contaminated bacteria in chicken meat samples. Resistant strain of *Salmonella enterica* serovar Kedougou isolated from chicken meat was used in this study. The outcome demonstrated that all bacteria cells in suspension and chicken meat samples were eradicated at 1 and 5 kGy, respectively. Bacterial CFUs were reduced corresponding to increasing doses of Gamma rays in both sample types. Survived bacteria exposed with 0.25 kGy of Gamma radiation did not show any changes of minimum inhibitory concentrations (MICs) value for ampicillin, tetracycline, and gentamicin. In summary, Gamma radiation at 1 and 5 kGy could be used for sterilization in both bacterial cell suspension and those contaminated in meat products. However, low dose of Gamma rays at 0.25 kGy, bacteria were not killed and still showed high level of antimicrobial resistance.

บทคัดย่อ

เชื้อแบคทีเรียดื้อยาต้านจุลชีพถูกพบปนเปื้อนอยู่ในอาหารหลายชนิด การฉายรังสีแกมมาเป็นหนึ่งในวิธีการฆ่าเชื้อแบคทีเรียในอาหาร การศึกษานี้มีจุดประสงค์เพื่อทดสอบผลของการฉายรังสีแกมมาต่อเชื้อแบคทีเรียดื้อยาซึ่งอยู่ในแบบเซลล์แขวนลอยและแบบของเซลล์ปนเปื้อนในเนื้อไก่ โดยใช้เชื้อซา*ลโมเนลลา* ซีโรวาห์คิโดกุซึ่งดื้อยาต้านจุลชีพในระดับสูงเป็นแบบจำลองในการทดสอบ ผลการทดลองพบว่า ปริมาณรังสีดูดกลืนที่ 1 และ 5 kGy สามารถฆ่าเชื้อแบคทีเรียที่แขวนลอยในอาหารเหลวและในเนื้อไก่ได้ตามลำดับ เซลล์แบคทีเรียมีจำนวนลดลงเมื่อเพิ่มปริมาณรังสีดูดกลืนในตัวอย่างทั้งสองชนิด แบคทีเรียแขวนลอยที่รอดชีวิตเมื่อนำมาทดสอบความไวต่อยาต้านจุลชีพพบว่า ค่าความเข้มข้น*ของ*ยาต่ำสุดที่สามารถยับยั้งเชื้อแบคทีเรีย (MIC) ต่อยา*แอมพิซิลลิน เตตระไซคลิน*และ*เจนตามัยซิน*ไม่มีการเปลี่ยนแปลง จึงสรุปได้ว่า การฉายรังสีแกมมาที่ 1 และ 5 kGy สามารถฆ่าเชื้อแบคทีเรียดื้อยาทั้งในสารละลายแขวนลอยและในเนื้อไก่ได้ แต่ปริมาณรังสีดูดกลืนที่ระดับต่ำเช่น 0.25 kGy พบว่ายังมีแบคทีเรียอยู่รอดและมีความสามารถในการดื้อยาต้านจุลชีพอยู่ในระดับสูงตามเดิม

Keywords: Antimicrobial resistant bacteria, Gamma irradiation, Decontamination

คำสำคัญ: เชื้อแบคทีเรียดื้อยา การฉายรังสีแกมมา การฆ่าเชื้อ

* *Student, Master of Science Program in Medical Microbiology, Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University*

** *Associate Professor, Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University*

Antimicrobial resistance (AMR) is one of the critical public health problems. The complexity of this scenario highly affects the worldwide population in all global regions. The mortality rate from this case increases every year. In Southeast Asia, a major contribution in this region is multidrug resistant Gram negative bacteria (MDRGN) (Suwantarat & Carroll, 2016). Southeast Asia is a highly dynamic region in terms of economic flow and epicenter of emerging AMR. Especially in Thailand, 38,481 patients died from AMR hospital acquired infection in 2010. (Zellweger et al., 2017) Antimicrobial resistant bacteria (ARB) can be contaminated in the environment, such as soil, water, and agricultural areas, etc. Thus, they can be transferred to the food system. When types of food are considered, chicken meat is one of animal products that should be low in resistant microorganism contamination due to less use of antibiotics for their growth. However, in Thailand, many studies were conducted and found resistant bacteria contamination in chicken meat, for example, in 2012 Chaisatit et al. (2012) found resistant *E.coli* and *Salmonella* spp. in chicken meat from Bangkok supermarkets. A study in 2005 could isolate resistant bacteria for tetracycline, amoxicillin, gentamicin from chicken samples in the local retail market in Khon Kaen Province, Northeastern of Thailand. (Angkititrakul et al., 2005)

Nowadays, various strategies are applied to eliminate bacterial contamination. (Sharma et al., 2016) However, the techniques for food should be safe, residual free and efficient to eliminate contaminant bacteria. Gamma irradiation is one of the sterile alternative techniques applied for food to be microbiologically sterile and extend the storability of food products. (Farkas, 2011) This strategy plays a crucial role in killing some food borne pathogens, such as *Salmonella*, *Staphylococcus aureus*, *Campylobacter*, *E. coli* O157: H7, etc. The dose of Gamma rays used in food depends on the type of food and objective of irradiation. For resistant bacteria, the susceptibility of survival bacteria after exposed with Gamma rays was also concerned. In previous study, non-lethal Gamma irradiation can effect on antimicrobial susceptibility of some *Salmonella* serotypes as shown in agar diffusion testing (Yahia et al., 2015). From all available information, there are only a few studies directly performed on highly resistant bacteria. Therefore, the study on the effect of this ionizing irradiation on resistant bacteria has been performed.

Objective of the study

The objective of this study was to determine the effect of Gamma irradiation on resistant bacterial suspension and resistant bacteria contaminated in chicken meat in term of viability, bacterial quantitation, and antimicrobial susceptibility.

Methodology

Bacterial strain preparation

Salmonella enterica serovar Kedougou or Sal162 isolated from chicken meat in Bangkok supermarket wasused in foodborne pathogen model. Antimicrobial resistance profile of this strain showed highly resistance for ampicillin, gentamicin and tetracycline drugs. Bacterial cells were recovered from -80˚C freezer and cultured on 5%

sheep blood agar (Clinag Co., Ltd., Thailand). Pure colonies were resuspended into sterile 0.85% NaCl solution (Merck & Co., Inc., USA) and adjusted until the suspension was equal to 0.5 McFarland.

Sample set

To determine the effect of Gamma irradiation, bacterial suspension and chicken meat samples were prepared. Bacterial cells in 0.5 McFarland suspension (*Sal*162) were transferred into new microcentrifuge tubes for suspension samples and 5 mL was spiked into 25 g of chicken meat samples placed in sterile stomacher bags (Amornwatana associates Part., Ltd, Thailand). The experimental set is shown in Figure 1.

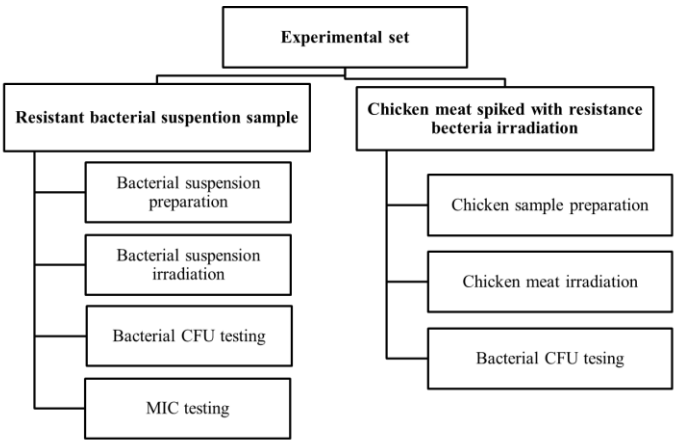

Figure 1 Sample sets used in this experiment

Gamma irradiation

Gamma irradiation process was conducted at Thailand Institute of Nuclear Technology: TINT, Nakhon Nayok province. Bacterial suspension and chicken meat samples were subjected to Gamma chamber machine (GC 5000, BRIT, India), a compact self-shielded Cobalt-60 (Co60). Five selected doses of Gamma rays were composed of 0.25, 1, 2, 5, and 10 kGy. All Samples were put into the sample chamber located in the vertical drawer inside the lead flask. After that, the drawer was moved down with the help of a system of motorized drive. Irradiation dose was completed by cumulative exposure. The rate of Gamma rays releasing was 2.75 kGy/hr. Therefore, time of irradiation was calculated and set for targeted doses.

Figure 2 Gamma chamber machine (GC5000)

The viability of bacterial cell

The viability of bacteria after irradiation was tested. One full loop of each suspension samples was streaked on 5% sheep blood agar plates and incubated at 37˚C for 16-18 hours. The viability of bacteria from each sample was observed from bacterial growth on those plates.

Bacterial colony forming unit (CFU) testing

Two hundred and twenty mL of buffer peptone water was added in chicken meat samples and homogenized by using stomacher machine (IUL S.A. Co.,Ltd., Spain) at high speed for 240 seconds. The homogenized solution (100 µl) was pipetted into 900 µl of BPW and prepared forserial tenfold dilution. One hundred microliters from each dilution was spread on plate count agar or PCA plates. For bacterial suspension sample, 100 µl of suspensions were tenfold serially diluted by NSS solution and spread on PCA plates.

Minimum inhibitory concentration of bacterial cell exposed to Gamma rays

Survived bacterial cells irradiated with Gamma rays were tested for MICs by broth microdilution. Antimicrobial agents selected for antibiogram were composed of ampicillin, tetracycline, and gentamicin. The process comprised three steps: antimicrobial agent preparation, bacterial inoculum preparation, and broth microdilution, which was conducted according to the 2015 CLSI guideline. (CLSI, 2015)

Results

For suspension samples, the viability of bacterial cells demonstrated that Gamma irradiation at least 1 kGy could kill bacteria in suspension form (Table 1). Thus, at 2, 5 and 10 kGy of radiation, there were no growth of bacteria on a culture plate. Numbers of bacterial CFU/mL of suspension samples are demonstrated in Table 1. The number of bacterial cells was decreased from 2.09×10^9 to 1.35×10^8 CFU/mL, when they were exposed with 0.25 kGy of irradiation. In Table 2, MIC result showed that all targeted doses of irradiation could not effect on MIC of highly resistance bacteria for amplicilin, tetracycline and gentamicin. For chicken meat samples, bacterial CFUs are shown in Table 3. When the dose of Gamma irradiation was increased, bacterial CFUs tended to drop down from approximately 10^{12} to 10^6 CFU/mL at 0 to 2 kGy, and no growth of bacteria was observed at 5 and 10 kGy.

Table 1 Viability of bacterial cells, bacterial CFUs and MIC of resistant bacterial suspension samples

Dose	Viability	Bacterial CFU	MIC		
			AM*	Gm*	Tet*
0 kGy	Growth (+)	2.09×10^{9}	1024	128	128
0.25 kGy	Growth (+)	1.35×10^{8}	1024	128	128
1 kGy	No growth (-)	0	**	**	**
2 kGy	No growth (-)	0	**	**	**
5 kGy	No growth (-)	0	**	**	**
10 kGy	No growth (-)	0	**	**	**

*AM=ampicillin, Gm = gentamicin, Tet = tetracycline

**No growth after irradiation

Table 2 Bacterial CFUs of chicken meat samples spiked with resistant bacteria

No.	Irradiation dose	Without irradiation	Bacterial CFUs after irradiation
1	0 kGy (control)	3.16×10^{12}	2.96×10^{12}
2	0.25 kGy	6.48×10^{13}	1.80×10^{11}
3	1 kGy	1.56×10^{13}	7.20×10^{6}
4	2 kGy	2.27×10^{13}	1.32×10^{6}
5	5 kGy	2.67×10^{13}	0
6	10 kGy	5.92×10^{12}	0

Discussion and conclusions

The results from this study could confirm that some doses of Gamma irradiation were effective to eradicate resistant bacteria, 1 kGy of Gamma rays was able to kill 0.5×10^{8}CFU/mL of bacteria in suspension form directly while the experiment of Mona M. K. Shehata et al. had to use 2.67 kGy to kill 3×10^{8} CFU/mL of *Staphylococcus aureus*. (M. Shehata, 2011) The dose difference between both experiments might be come from different composition of cell wall and concertation of bacteria. The data of this scheme might be applied for resistant bacteria decontamination in the clinical, agricultural and environmental fields. In practice, a dose of radiation was adjusted depend on bacterial concentration in the target area. When all resistant bacteria were eliminated, the dissemination of AMR will be reduced because of no bacterial load and decreasing chances of transfer of resistance genes via conjugation that required viability of bacteria (von Wintersdorff et al., 2016). A low dose of gamma rays in this experiment at 0.25 kGy could not get rid of all bacterial cells and survived bacteria exposed with that dose still had high resistance for antimicrobial agents. The outcome of MIC testing could infer that elimination of resistant bacteria could not be conducted in some part of the population since the left cells still showed the original traits, and irradiated

cells or weakened bacteria did not change MIC values. Our results contrasted with the previous study that described the effect of Gamma on the antimicrobial susceptibility. (Shokier et al., 2010) The explanation might be the different of irradiation bacterial form and method to measure antimicrobial susceptibility.

For chicken meat spiked with resistant bacteria. Five kGy of Gamma rays could kill all contaminated bacteria. Chicken meat samples required a higher dose than bacterial suspension samples, because other components in chicken such as protein and chicken skin might barriers and limited the target dose accession of Gamma rays. The number of bacterial cells in form of bacterial CFU/mL after irradiated showed reduction when radiation dose was increased. The suitable dose of Gamma rays to eliminate resistant bacteria must be figured out. For chicken meat and poultry product, the irradiation doses depend on the product forms. For example, frozen poultry use 3-5 kGy (Ministry of Public Health, Thailand, 1979), but chilled poultry use 1.5 – 2.5 kGy. (Ministry of Public Health, Thailand, 2006) Maximum of dose rate allowed to apply in Thailand announced by the ministry of public health is 10 kGy. (World Health Organization [WHO], 1981) However, the outcomes of irradiation were also important, such as nutrition losses, taste and physical changes. (Yoon, 2003). In this experiment, the dose rate of Gamma rays approximately 5 kGy would be suitable for chicken meat samples in case of resistant or true pathogenic bacteria contamination such as *Salmonella*. However, for some bacteria that are allowed to be present at permissible level, such as *E.coli* and coliform, etc., the optimal dose could be 1-2 kGy to reduce the number to the acceptable range (Department of Medical Sciences, Thailand, 2010)

Finally, Gamma irradiation can be one choice of application that is safe, efficient, environmentally clean, and energy efficient process. (Farkas, 1998)For further studies, the combination of Gamma rays with other application such as UV radiation and biodegradation, should be studied to solve some limitations of Gamma rays. The effects on DNA and resistance genes were also significant, because DNA and resistance genes still existed after bacterial cells were killed. So, transfer rate and gene function should be further investigated.

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