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Effects of gamma irradiation on the propagation of microbial growth in commonly available meat in Bangladesh

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Abstract

The present work endeavoured to emphasise on the huge proliferation of drug resistant microbial strains in common meat samples including pigeon, duck, quail, domestic chicken and broiler chicken. The present work also focused on the irradiation method for controlling the pathogenic microbes in five categories of 30 meat samples collected from food markets in Dhaka, Bangladesh. Half of the samples (non-irradiated) of each were prepared for microbiological profiling and the rest of the samples were gamma irradiated (6 kGy and 8 kGy). The resistant pattern of the isolates was also observed against 12 antibiotics. For non-irradiated samples, the heterotrophic bacterial and fungal loads were 1.5×10^8 CFU/g, and the faecal contamination was 10^6 CFU/g. Several pathogens like *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Vibrio* spp. and *Listeria* spp. were found up to 10^7 CFU/g . These isolates were found to be resistant against single or multiple antibiotics. Meanwhile, the efficacy of gamma-irradiation was outstanding on the growth of microorganisms; up to 4 log reduction was observed at 6 kGy, and 6 log reduction at 8 kGy. 6 kGy and 8 kGy were found to be very effective against multi drug resistant strains.

Introduction

As protein-rich foods, beef, mutton, lamb and chicken are extremely popular around the globe (Kanatt *et al*., 2015; Mohamed and El-Deen, 2016). Besides fresh meats, other meat products are also gaining popularity due to their high nutritive value and taste (Pelicia *et al*., 2015). In Bangladesh, poultry provides around 35.25% of the total meat consumption, and is gradually increasing (Begum *et al*., 2011; Akbar *et al*., 2013; Kanatt *et al*., 2015). Fresh meat and meat products naturally have very short shelf life, and their freshness greatly depends on varying factors including atmospheric oxygen (O_2), enzyme activity, holding temperature, light, moisture, and most importantly, microorganisms (Zhou *et al*., 2010; Kanatt *et al*., 2015).

Meat is also naturally an ideal habitat for the growth of several spoilage microorganisms such as *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. (Shaltouta *et al*., 2016). Lack of good manufacturing practice, poor sanitary condition while slaughtering and inappropriate preservation technologies enhance the risk of contamination and deterioration of fresh meats (Sánchez-Escalante *et al*., 2001; Pelicia *et al*., 2015; Kanatt *et al*., 2015). Several decontaminating procedures could be applied to prolong meats' shelf life such as freezing, cooking, salting, fermenting, smoking, picking and drying (Al-Sheddy *et al*., 1999; Kalalou *et al*., 2004). However, some of these methods are time- and energy-consuming.

Irradiation by ionising radiation has emerged as a safe, efficient, environmentally clean and energy-efficient process for eliminating microbial contamination while maintaining the freshness and nutritional quality of meat (O'Bryan *et al*., 2008; Arvanitoyannis *et al*., 2009; Noor *et al*., 2013; Acharjee *et al*., 2014a). Gamma ray is a short wavelength ionising radiation produced from radioactive isotopes cobalt 60 (60 Co) or caesium 137 (137 Cs) which could fatally damage the cell membrane and DNA of spoilage-causing microorganisms (IAEA, 2002).

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Typical radiation doses applied for decontamination process ranges between 2 to 8 kGy, which was also found to be effective against parasites (WHO, 1981; Pelicia *et al*., 2015). The performance of gamma-irradiation in eliminating foodborne pathogens and improving shelf life of varying meat products such as chicken, beef, beef, pork, lamb, rabbit and camel has been well documented (Ahn *et al*., 2004; Nam *et al*., 2004; Badr, 2004; Kanatt *et al*., 2006; 2007; Al-Bachir *et al*., 2009; Zhou *et al*., 2010; Noor *et al*., 2013; Acharjee *et al*., 2014a). The present work was therefore designed to determine the efficacy of gamma-irradiation in microbial inhibition of meats from five domestic fowls, namely, domestic chicken, broiler chicken, duck, pigeon and quail, as well as to demonstrate the drug resistant traits of pathogenic proliferation in these meat samples.

Materials and methods

Study area and sampling

Meat samples of domestic chicken (n = 6), broiler chicken (n = 6), duck (n = 6), pigeon (n = 6) and quail (n = 6) were randomly obtained from different food markets in Dhaka, Bangladesh from Jan to Apr 2017. The meat samples were aseptically collected early in the morning, and immediately transported to the laboratory by using sterile polyethylene bags with ice (Noor *et al*., 2013; Acharjee *et al*., 2014a).

Sample processing, irradiation and microbiological analysis

The meat samples were appropriately weighed, cut into three pieces, washed with distilled water, and placed in separate sterile polyethylene packets. For the three pieces, two were subjected to 6 kGy and 8 kGy gamma irradiation (provided by Board of Radiation and Isotope Technology, India) for 20 min, and properly sealed (Acharjee *et al*., 2014a). The remaining one piece (non-irradiated) was aseptically introduced for pathogenic study. Next, the packets containing both fresh (non-irradiated) and irradiated meat samples were washed with peptone buffer water, and each piece of irradiated meats was homogenised with normal saline (Noor *et al*., 2013; Acharjee *et al*., 2014a). The samples were then serially diluted up to 105.

Total viable bacteria (TVB), total faecal coliform (TFC), staphylococcal and fungal load estimation

For bacteriological profiling, all the samples were prepared, and spread-plate technique was performed. For the total viable bacteria (TVB), total faecal coliform (TFC), fungal count and *Staphylococcus aureus* count; 0.1 mL suspension from each dilution factor of the meat samples was spread onto Nutrient agar, Membrane Faecal Coliform (mFC) agar, Sabouraud Dextrose Agar (SDA) and Mannitol Salt Agar (MSA) plates, respectively (Md. Rokibul *et al*., 2013; Acharjee *et al*., 2014a). For TVB and *Staphylococcus aureus* count, plates were incubated at 37°C for 24 h, while for mFC, plates were incubated at 44.5°C for 24 h. The SDA plates were incubated at 25°C for 48 h (Acharjee *et al*., 2014a).

Isolation of Salmonella spp., Shigella spp., Vibrio spp., Pseudomonas spp. and Listeria spp.

For the determination of viable but non culturable microorganisms, the enrichment methods were performed (Colwell, 2000; Oliver, 2010). Briefly, 1 mL of each samples were added to selenite cysteine broth (SCB) and alkaline peptone water (APW), and incubated at 37°C for 6 h, and then 0.1 mL suspension was spread onto *Salmonella Shigella* (SS) agar and Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar, for the assay of *Salmonella* spp., *Shigella* spp., and *Vibrio* spp., respectively. For the isolation of *Listeria* spp. and *Pseudomonas* spp., 0.1 mL suspension was spread onto *Listeria* identification media and Cetrimide agar, respectively. Next, the inoculated plates were incubated at 37°C for 24 h. Finally, the confirmation of all the isolates were examined by the standard biochemical tests (Cappuccino and Sherman, 1996; Acharjee *et al*., 2014a; 2014b).

Determination of antimicrobial susceptibility

For the examination of the efficacy of different synthetic drugs against different resistant strains, the Kirby-Bauer or disc diffusion method is more consistent and popular (Bauer *et al*., 1966; Ferraro, 2001). Twelve antibiotics were used: ampicillin (AMP, 10 µg), tetracycline (TER, 30 µg), imipenem (IPM, 10 µg), azithromycin (AZI, 15 µg), penicillin (PEN, 10 µg), gentamicin (GEN, 10 µg), streptomycin (STP, 10 µg), erythromycin (ERY, 15 µg), ciprofloxacin (CIP, 5 µg), ceftriaxone (CEF, 30 µg), cefixime (CFX, 5 µg) and chloramphenicol (CHL, 10 µg), against the isolated pathogens. The strains' resistance (R) or sensitivity/susceptibility (S) was determined through the disc-diffusion assay on Müeller-Hinton agar (Difco, Detroit, MI) (Bauer *et al*., 1966; Ferraro, 2001, Munshi *et al*., 2012; Acharjee *et al*., 2014a; 2014b).

Statistical analysis

All experiments were performed three times. Statistical analyses were performed by determining the p-value through t-test. Errors were also calculated.

Results and discussion

Due to the poor storage condition and unhygienic environment, huge amount of foods and food products are affected on their quality, nutritive value and marketability day by day (Noor *et al*., 2013; Acharjee *et al*., 2014a). Several microorganisms such as *E. coli*, *Salmonella* spp., *Staphylococcus* spp., *Listeria* spp., and *Campylobacter jejuni*, are very common contaminants in meats and meat products (Ahmad *et al*., 2013; Saleem *et al*., 2015). Irradiation is one of the best and effective methods which can eliminate the potential pathogens in different food items especially in fish and meat products (Acharjee *et al*., 2014a; Shaltouta *et al*., 2016).

Occurrence of pathogenic microorganisms before irradiation

Huge microbial contamination was found in all meat samples especially in non-irradiated samples (Figure 1). Both packed washed water and meat blends exhibited huge array of heterotrophic bacteria, fungi and total faecal coliform within the range of 10^5 to 10^8 CFU/g. Several pathogens like *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Vibrio* spp. and *Listeria* spp. were found within the range of 10^2 to 10^7 CFU/g in both packed washed water and meat blend samples. Among all the pathogens, *Staphylococcus* spp. was predominant in both cases (Figure 1). All the pathogenic isolates were biochemically identified (Table 1).

For pigeon samples, both packets washed water and meat blend yielded *Shigella* spp., *Vibrio* spp., *Staphylococcus* spp., *Pseudomonas* spp. and *Listeria* spp. while the growth of *Salmonella* spp. was absent in both cases (Figures 1A and 1B).

For duck samples, the packed washed water and meat blend yielded *Salmonella* spp., *Vibrio* spp., *Staphylococcus* spp. and *Listeria* spp. up to 10^6 CFU/g, whereas only *Pseudomonas* spp. was found in meat blend. *Shigella* spp. was absent in both cases (Figures 1C and 1D).

For quail samples, the packed washed water and meat blend yielded *Shigella* spp., *Vibrio* spp., *Staphylococcus* spp. *Pseudomonas* spp. and *Listeria* spp. up to 10^7 CFU/g, whereas *Salmonella* spp. was found only in packed washed water. Growth of faecal coliform was absent in both cases (Figures 1E and 1F).

For domestic chicken samples, the packed washed water and meat blend yielded *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Staphylococcus* spp. and *Pseudomonas* spp. up to 10^6 CFU/g . *Listeria* spp. was absent in both cases (Figures 1G and 1H).

For broiler chicken samples, the packed washed water and meat blend yielded *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Pseudomonas* spp. and *Listeria* spp. within the range of 10^2 to 10^6 CFU/g. *Vibrio* spp. was absent in both cases (Figures 1I and 1J).

Mohamed and El-Deen (2016) stated that the propagation of *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. was very predominant in meat samples which might destroy the meat quality as well as increase the risk of foodborne diseases (Md. Rokibul *et al*., 2013; Noor *et al*., 2013; Acharjee *et al*., 2014a; 2014b). The possible sources of such contamination may arise from storage condition when food came into contact with water and ice.

Effect of gamma-irradiation (6 kGy and 8 kGy) on pathogenic reduction

Following irradiation, huge number of pathogenic loads were eliminated in both packet washed water and meat blend samples (Figure 1). Some of the pathogens were found to be completely eliminated (100%) after irradiation.

For pigeon samples, the total viable bacteria, faecal coliform and fungi were found to be reduced within the range of 2 to 3 log at 6 kGy in both packet washed water and meat blend, while the load was reduced within the range of 4 to 6 log at 8 kGy. Only faecal contamination was totally eliminated in meat blend at 8 kGy (Figures 1A and 1B). In packet

Table 1. Biochemical identification of different pathogenic isolates found in different meat samples.

Isolates	TSI			H_2S reaction	Indole test	MR test	VP test	Citrate test	Motility test	Oxidase test
	Slant	Butt	Gas							
Salmonella spp.	R	Y	-	+	-	+	-	-	+	-
Shigella spp.	R	Y	-	-	+/-	+	-	-	-	-
Vibrio spp.	Y	Y	-	-	+	+	-	+	+	+
Staphylococcus spp.	Y	R	+	+	-	+	-	+	+	-
Listeria spp.	Y	Y	-	-	-	+	+	-	+	-
Pseudomonas spp.	R	R	-	-	-	-	-	+	+	+

The experiments were conducted three times independently, and the results were found to be reproducible. One representative data has been shown.
TSI = Triple Sugar Iron Test, Y = yellow (acid), R = red (alkaline), MR = Methyl red, VP = Voges-Proskauer.

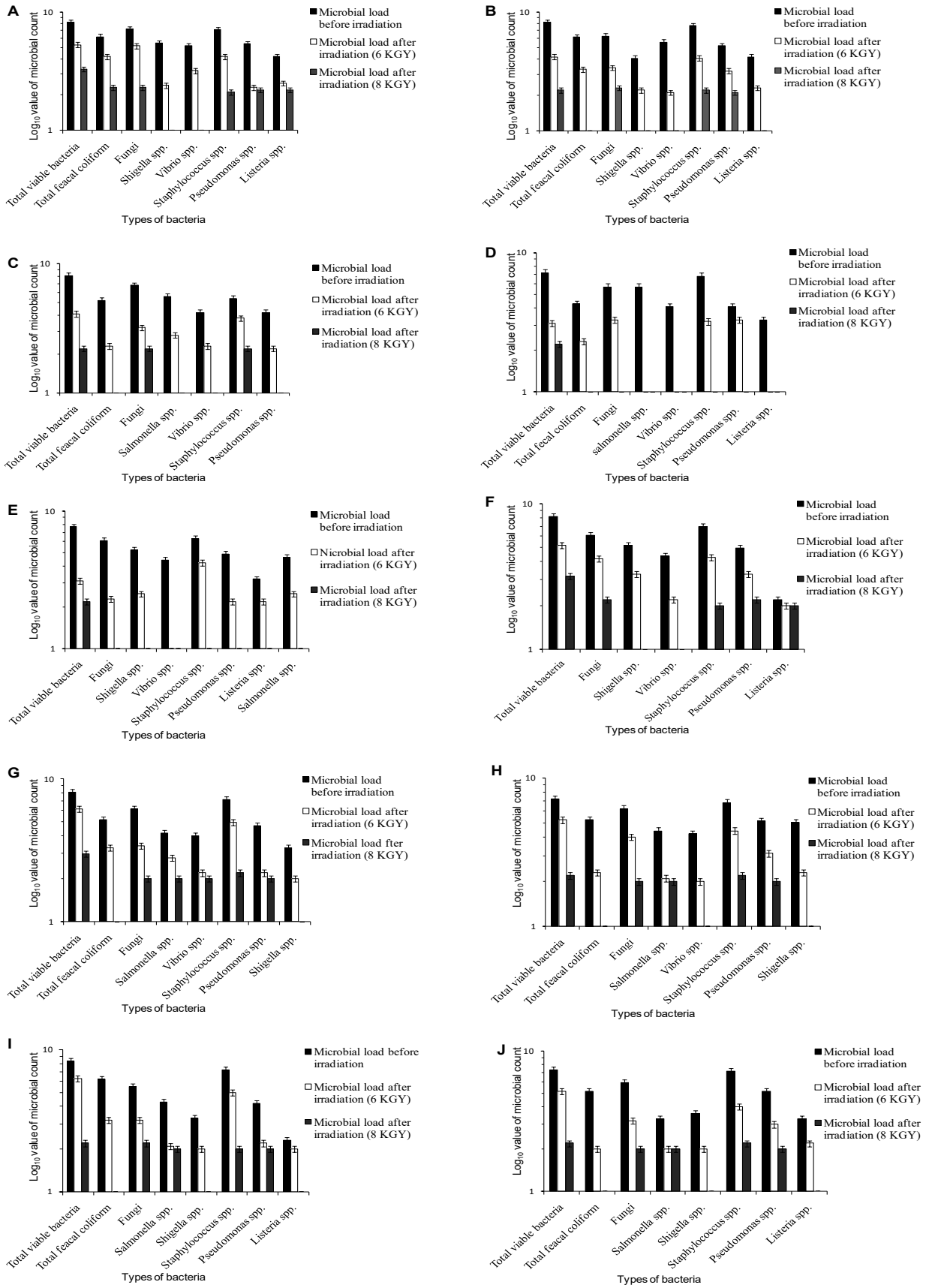

Figure 1: Effect of gamma irradiation on the reduction of microbial pathogenic load. [A-B] for pigeon, [C-D] for duck, [E-F] for quail, [G-H] for domestic chicken, and [I-J] for broiler chicken samples. A, C, E, G and I: packet washed with water samples; B, D, F, H and J: meat blend samples. White bars and light back indicate the irradiated samples (6 kGy, 8 kGy), while black bars denote the non-irradiated samples.

washed water and meat blend, the growth of all pathogens was eliminated up to 3 log at 6 kGy, and 5 log at 8 kGy. Only *Vibrio* and *Shigella* spp. showed 100% elimination at 8 kGy in packed washed water, and *Shigella* spp., *Vibrio* spp. and *Listeria* spp. were completely (100%) eliminated at 8 kGy in meat blend (Figures 1A and 1B).

For duck samples, the reduction rate was noticed up to 3 log for total viable bacteria, faecal coliform and fungi in both packets washed water and meat blend at 6 kGy. At 8 kGy, the reduction rate was found within the range of 1 to 6 log for packet washed water, and 1 to 5 log for meat blend. In both cases the *Salmonella* spp. and faecal coliform were totally eliminated at 8 kGy (Figures 1C and 1D).

For quail samples, the reduction rate was up to 3 log for total viable bacteria and fungi at 6 kGy, and up to 5 log at 8 kGy in both packets washed water and meat blend. The pathogens were found to be eliminated up to 2 log at 6 kGy and 4 log at 8 kGy in packet washed water. For meat blend, the reduction rate was calculated up to 3 log at 6 kGy, and 5 log at 8 kGy. In both cases, *Vibrio* and *Shigella* spp. were completely eliminated (100%) at 8 kGy (Figures 1E and 1F).

For broiler chicken samples, the reduction rate was up to 3 log for total viable bacteria, faecal coliform and fungi at 6 kGy in packet washed water, while up to 4 log at 6 kGy in meat blend (Figures 1G and 1H). At 6 kGy, all the pathogens were found to be reduced up to 2 log in both packet washed water and meat blend, while most of the pathogens including faecal coliform and fungi were found to be completely (100%) eliminated at 8 kGy (Figures 1G and 1H).

For broiler chicken samples, the reduction rate was up to 3 log for total viable bacteria, faecal coliform and fungi at 6 kGy in packet washed water, while *Salmonella* spp., *Shigella* spp. and *Listeria* spp. were completely (100%) eliminated. *Staphylococcus* spp. and *Pseudomonas* spp. were reduced 4 log and 2 log respectively (Figures 1I and 1J). At 8 kGy, all the microflora were completely (100%) eliminated except for *Staphylococcus* spp. For meat blend, faecal coliform, *Salmonella* spp., *Shigella* spp. and *Listeria* spp. showed no growth at 6 kGy and at 8 kGy (Figures 1I and 1J).

The growth of several Gram-negative pathogens such as *E. coli*, *Staphylococcus* spp. and *Salmonella* in meat could significantly reduce meat's shelf life and texture quality (Pelicia *et al*., 2015; Kanatt *et al*., 2015; Shaltouta *et al*., 2016). Mohamed and El-Deen (2016) reported that the effects of different irradiation doses on the acceleration of bacterial growth was very high especially on Gram-negative bacteria. The present work showed the growth of bacteria was significantly eliminated at 6 kGy and 100% reduction was observed at 8 kGy for most pathogens. Nearly similar results were reported on sea fish samples (Acharjee *et al*., 2014a).

According to Food and Agricultural Organization (FAO) and World Health Organization (WHO), irradiation at doses up to 10 kGy could be accepted as a process for food preservation without any special nutritional problem (IAEA, 2002; Ibrahim *et al*., 2013). Currently, more than 60 countries are using this irradiation process on a commercial scale as a decontaminating method of foods (Tauxe, 2001). According to the Centres for Disease Control and Prevention (CDC), in United States, irradiation of half or all ground pork, beef, poultry and processed meat would decrease the foodborne disease cases by one million and prevent about 350 deaths by inactivating major foodborne pathogens including *Pseudomonas*, *Salmonella*, *E. coli* O157:H7, *Campylobacter*, *Listeria*, lactic acid bacteria and others (Mulder *et al*., 1977; Chinen *et al*., 2001).

Existence of drug-resistant pathogens in different meat samples

The development of resistant genes in disease-causing bacteria is a global concern (Md. Rokibul *et al*., 2013; Noor *et al*., 2013; Acharjee *et al*., 2014a; 2014b). The present work found several resistant strains against different drugs. The meat samples were found to harbour 100% resistant strains of *Listeria* spp. against AMP, CIP, STE, PEN, TER, CHL CEF, IPM, GEN, AZI, CFX and ERY. *Vibrio* spp. exhibited 100% resistance against AMP, STE, PEN, TER and 100% sensitive/susceptible against CIP, CEF, IPM, GEN, AZI, CFX, ERY and CHL. *Staphylococcus* spp. exhibited 100% resistance against AMP, CIP, STE, PEN, TER, CEF, IPM, GEN, AZI, CFX, ERY and CHL. *Salmonella* spp. was found 100% resistance against AMP, CIP, STE, CEF, PEN and 100% sensitive/susceptible against IPM, GEN, AZI, TER, CFX, ERY and CHL. *Pseudomonas* spp. showed 100% resistance against all the drugs AMP, CIP, STE, PEN, TER, CEF, IPM, GEN, AZI, CFX, ERY and CHL. *Shigella* spp. was found to be resistant against AMP, CIP, STE, PEN, TER and 100% sensitive/susceptible against CEF, IPM, GEN, AZI, CFX, ERY and CHL (Table 2). One of our previous works found resistant bacteria against more than one antibiotic from different fish samples (Noor *et al*., 2013; Acharjee *et al*., 2014a). They found only gentamycin (10 µg) as effective drug against most bacterial strains while the present work demonstrated

Table 2. Antibacterial susceptibility pattern of different pathogenic isolates found in different meat samples.

Antibiotic	Listeria spp.		Vibrio spp.		Staphylococcus spp.		Salmonella spp.		Pseudomonas spp.		Shigella spp.	
	R	S	R	S	R	S	R	S	R	S	R	S
AMP (10 µg)	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%
CIP (5 µg)	100%	0%	0%	100%	100%	0%	100%	0%	100%	0%	100%	0%
STE (10 µg)	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%
CEF (30 µg)	100%	0%	0%	100%	100%	0%	100%	0%	100%	0%	0%	100%
IPM (30 µg)	100%	0%	0%	100%	100%	0%	0%	100%	100%	0%	0%	100%
PEN (10 µg)	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%
GEN (10 µg)	100%	0%	0%	100%	100%	0%	0%	100%	100%	0%	0%	100%
AZI (15 µg)	100%	0%	0%	100%	100%	0%	0%	100%	100%	0%	0%	100%
TER (30 µg)	100%	0%	100%	0%	100%	0%	0%	100%	100%	0%	100%	0%
CFX (5 µg)	100%	0%	0%	100%	100%	0%	0%	100%	100%	0%	0%	100%
ERY (15 µg)	100%	0%	0%	100%	100%	0%	0%	100%	100%	0%	0%	100%
CHL (10 µg)	100%	0%	0%	100%	100%	0%	0%	100%	100%	0%	0%	100%

AMP = ampicillin, CIP = ciprofloxacin, STE = streptomycin, CEF = ceftriaxone, IPM = imipenem, PEN = penicillin, GEN = gentamicin, AZI = azithromycin, TER = tetracycline, CFX = cefixime, ERY = erythromycin, CHL = chloramphenicol, n = number of isolates, R = resistant, S = sensitive / susceptible.

that the isolated *Staphylococcus* spp. *Pseudomonas* spp. and *Listeria* spp. from meat samples were found to be 100% resistant against gentamycin (10 µg) and streptomycin (10 µg). Huge propagation of such resistant strains in meats is not a good sign for public health and trade (Bennett, 2008; Canteón, 2009; Acharjee *et al*., 2014a).

Conclusion

The present work successfully concluded that the presence of pathogenic microbes in meat samples remains serious public health risk. According to the present findings, researchers can easily state that the irradiation is very effective method for controlling the growth of microorganisms as well as a very economical rather than the other decontaminating methods. The growth of bacteria and fungi were found in very low amount at 6 kGy and 8 kGy. Even the multidrug resistant (MDR) strain was also found to be eliminated following gamma-irradiation. However, to eradicate such pathogenic contaminants from meats and meat products, proper sanitation during handling, packaging, storage and transportation should be implemented which would decrease the consumer's health risk and extent the meat shelf life.

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