

## Consequences of $\gamma$ -Irradiation on the Dissemination of Microorganisms among Sea Fish in Bangladesh

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### Abstract

In cohort with our earlier investigation, present study attempted to evaluate the existence and survival of spoilage microorganisms in five common sea fishes (*Scomber colias*, *Lates calcarifer*, *Tenulosa ilisha*, *Pandalus borealis*, *Colossoma macropomum*) available in Bangladesh and also optimized the doses of  $\gamma$ -irradiations to reduce the proliferation of the bacterial pathogens. Total 75 samples of 5 categories ( $n = 15$ ) sea fishes were collected from the super shops in Dhaka city; and each sample was subjected to  $\gamma$ -irradiation (6 kGy and 8 kGy). Both samples (non-irradiated and irradiated) were analyzed for the existence of pathogenic bacteria through the conventional cultural techniques and the confirmative biochemical identification procedures. Response of the microbial isolates against different drugs was also investigated. Most of the non-irradiated samples were found to harbor a huge population of microorganisms up to  $1.5 \times 10^8$  cfu/g or cfu/mL including the fecal coliforms ranging up to  $10^6$  cfu/g or cfu/mL. Several specific bacterial species like *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Vibrio* spp. and *Listeria* spp. were noticed to be present in a titer of  $10^7$  cfu/mL or cfu/g. Moreover, the identified isolates were found to be resistant against single or multiple antibiotics tested. Thus, the incidence of coliforms and other harmful bacteria together with their drug-resistance traits may be considered as a serious threatening to the public health upon consumption of such fishes. When subjected to  $\gamma$ -irradiation, the propagation rate of pathogens was reduced up to 4 logs with the irradiation dose of 6 kGy; however, 100% reduction was observed when 8 kGy was applied.

**Keywords:** Gamma ( $\gamma$ ) Irradiation; 6 kGy and 8 kGy; Sea Fish; Pathogens; Antibiotic Resistance; Microbiological Quality

### Introduction

Fish is one of the leading aquatic organisms serving as the major food component of human and other animals around the world [1,2]. Fisheries sector plays a significant role in food security, economical growth and poverty alleviation in Bangladesh as it contributes around 60% of the total national demand for animal protein [3-5] and also contributes the global export market as well [5,6]. However, due to its highly perishable nature, every year a huge amount of fish is spoiled in Bangladesh which in turn pose a great threat to the export market [5-8]. Previous studies together with the local researches showed that fish and fish products are highly susceptible to varying types of pathogenic and potentially pathogenic microorganisms including *Vibrio cholerae*, *Shigella* spp., *Listeria monocytogenes*, *Clostridium botulinum*, *Salmonella* spp., *Campylobacter*, *Staphylococcus aureus*, etc. [4,8,9]. Moreover, this is to be noted that sea fishes are highly susceptible to surface or tissue contamination by microorganisms originating from their marine environment [4,6,10].

A number of reports has been published on the sea fishes which were contaminated with pathogenic bacteria during harvesting, transportation, storage and also due to the poor establishment of the good manufacturing practices (GMPs) [6,11,12]. Therefore, the food borne disease outbreaks may occur due to the consumption of microbiologically spoiled fish and the consumer acceptability may largely be affected for the off-odor and off-taste of the products by rancidity [13-15]. Furthermore, the extensive and widespread use of antibiotics in agriculture sectors as well as in aquaculture systems may act as a potential source of antibiotic resistant bacteria in fish sample [5,16-18].

In order to minimize the microbial contamination within fishes, several techniques are traditionally used for the preservation of fish among the developing countries including in Bangladesh such as cold storage, rapid chilling, freezing, smoking, heating, organic acids, antimicrobials, antioxidants edible coating and modified atmosphere packaging etc [4,19-22]. Besides conventional techniques of fish preservation, ionizing radiation has long been known as a cost and energy effective procedure to extend the shelf life of fish and fishery products; however, the implication of the irradiation technology to reduce microbial count within fish is still scarce in Bangladesh [23-27]. World Health Organization (WHO) and Food and Agricultural Organization (FAO) approved that irradiation doses up to 10 kGy could be used for decontamination of food without any significant nutritional problem [23,24]. Hence considering the popularity of fish as the foodstuff, nutritional importance of fish, and above all as a major export item of Bangladesh, it is necessary to maintain the microbiological quality of the sea fish as well as to establish the preventive mechanism which can eliminate the microbial growth and ensure the public health safety.

Our earlier research on irradiating fish samples (*Pseudapocryptes elongatus*, *Scomberomorus cavalla*, *Xenentodon cancila* and *Otolithoides pama*) applying a dose of 3 kGy to achieve microbiological quality demonstrated an average reduction of microorganisms by 3-log [25]. To achieve the total reduction of microorganisms among the fishes, present study was further designed not only to detect the microbial prevalence and drug resistant pathogens but also to establish the efficacy of ionizing radiation ( $\gamma$ -irradiation) of varying doses (6 kGy and 8 kGy) for the commercial supply of popular fishes free of microbial contamination.

## Material and Methods

### Study area and sampling

Total 75 samples of 5 categories fishes were studied. Fifteen (15) samples of *Scomber Colias* (chub mackerel), 15 *Lates calcarifer* (Koral), 15 *Tenualosa Ilisha* (hilsha), 15 *Pandalus Borealis* (shrimp), 15 *Colossoma macropomum* (rupchanda) were collected from different supper shop in Dhaka city within a time frame of January, 2017 to April, 2017. All the samples were collected aseptically and transported immediately to the laboratory by using sterile polyethylene bags with ice [3,4,25].

### Sample processing, irradiation and microbiological analysis

The appropriate length and weight of the each fish sample were measured and cut into 3 pieces and then the samples were washed out with distilled water. Each piece of samples were put in separate sterile polythene packets (irradiation by at 15 kGy), and sealed properly [25]. Two pieces of the samples were irradiated separately at 6 kGy and 8 kGy using 60 cobalt radiation source (provided by Board of Radiation and Isotope Technology, India) for 20 minutes, and the remaining half (i.e., non-irradiated) were subjected to pathogenic study [25]. Then the packets of both fresh (non-irradiated) and irradiated samples were washed with peptone buffer water and each piece of both irradiated fish was homogenized with normal saline [25]. The packet washed water and the fish blend samples were then serially diluted up to  $10^{-5}$  for microbiological analysis [4]. As described earlier [25], according to the information provided by the Institute of Food and Radiation Biology (IFRB), Bangladesh Atomic Energy Commission (BAEC), Dhaka, Bangladesh, current study implemented gamma irradiation on different sea fish samples (Supplement 1).

### Estimation of total viable bacteria (TVB), total fecal coliform (TFC), staphylococcal and fungi

In order to detect the presence (as well as the quantification) of the total viable bacteria (TVB), total fecal coliform (TFC), Fungal count and *Staphylococcus aureus*, 0.1 ml of suspension from each dilution of the samples was spread out onto Nutrient agar, Membrane Fecal Coliform (mFC) agar, Sabouraud Dextrose Agar (SDA) and Manitol Salt Agar (MSA) plates, consecutively [3,29]. For TVB and Staphylococcal assay, plates were incubated at 37°C for 24 hours while for estimating the fecal coliforms, plates were incubated at 44.5°C for 24 hours. The SDA plates were incubated at 25°C for 48 hours for the determination of fungal count.

### Detecting the presence of *Salmonella* spp., *Shigella* spp., *Vibrio* spp. *Pseudomonas* spp and *Listeria* spp.

Since *Salmonella* spp., *Shigella* spp., *Vibrio* spp. *Pseudomonas* spp and *Listeria* spp. may survive in the natural environment (for example, within the fish samples tested) but their presence can be often misguided by their viable but nonculturable (VBNC) state and thereby imparting false negative results *in vitro* [30-32]. The fish samples were further subjected to enrichment for the isolation and identification of such VBNC bacteria [33-35]. One ml of each samples were added to selenite cystein broth (SCB) and alkaline peptone water (APW) and incubated at 37°C for 6 hours, and then 0.1 ml of suspension was spread onto *Salmonella Shigella* (SS) agar and Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar media, for the assay of *Salmonella* spp., *Shigella* spp., and *Vibrio* spp., consecutively. For the isolation of *Listeria* spp. and *Pseudomonas* spp., 0.1 ml of suspension was spread onto *Listeria* identification media and Cetrimide agar respectively. Then the plates were incubated at 37°C for 24 hours. Finally, the confirmation of all the isolates was examined by the standard biochemical tests [25,29,31].

### Statistical analysis

All the experiments were performed in three times. Statistical analyses were performed by determining the p-value through *t* test. Errors were also calculated [25,36].

### Determination of antimicrobial susceptibility

The pathogenic isolates were examined for antibiotic susceptibility traits (either drug resistant or sensitive) by disc diffusion assay on Mueller-Hinton agar (Difco, Detroit, MI) against commonly used 12 antibiotics following the standard protocol [37-40]. Antibiotics used in the study included Ampicillin (AMP 10  $\mu\text{g}$ ), Tetracycline (TER 30  $\mu\text{g}$ ), Imipenem (10  $\mu\text{g}$ ), Azithromycin (AZI 15  $\mu\text{g}$ ), Penicillin (PEN 10  $\mu\text{g}$ ), Gentamicin (GEN 10  $\mu\text{g}$ ), Streptomycin (STP 10 $\mu\text{g}$ ), Erythromycin (15  $\mu\text{g}$ ), Ciprofloxacin (CIP 5  $\mu\text{g}$ ), Ceftriaxone (CEF 30  $\mu\text{g}$ ), Cefixime (CFX 5  $\mu\text{g}$ ) and Chloramphenicol (CHL 10  $\mu\text{g}$ ).

## Results and Discussion

Contamination of sea fish by bacteria and fungi is not unlikely posing a fatal impact on the global food safety as well as on the public health. One of our previous studies showed the presence of a bulk amount of microorganisms within sea fish samples of which most were also noticed to be drug-resistant [4]. In order to minimize these microbial populations, a simulation experiment employing  $\lambda$ -irradiation technology was lunched thereafter [25]. Fortunately, the pathogenic load was found to be significantly declined upon a dose of 3 kGy as stated earlier [25]. The current study, even apparently appearing as an increment of the previous work, the novelty actually underlies the 100% reduction of microorganisms within the irradiated fish samples subjected to dose of 8 kGy. Thus, the present work goes much to deal with the regulation of food safety; and also may aid to the understanding of food protection by the appropriate identification of fish contamination microorganisms as well as their eradication.

### Prevalence of pathogenic microorganisms before $\gamma$ -irradiation

All the 5 categories of 75 sea fish unveiled the existence of bacterial pathogens including fecal coliforms, *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Vibrio* spp. and *Listeria* spp. (Table 1). The total viable bacteria was found up to 10<sup>8</sup> cfu/ml or cfu/g and the fungal growth peaked up to 10<sup>7</sup> cfu/ml or cfu/g. in both packet washed water and within the fish blend for 5 categories of

sea fishes. It was noticed that the microbial contamination was higher in the fish washed water than those in the fish blends in all samples (Figure 1).

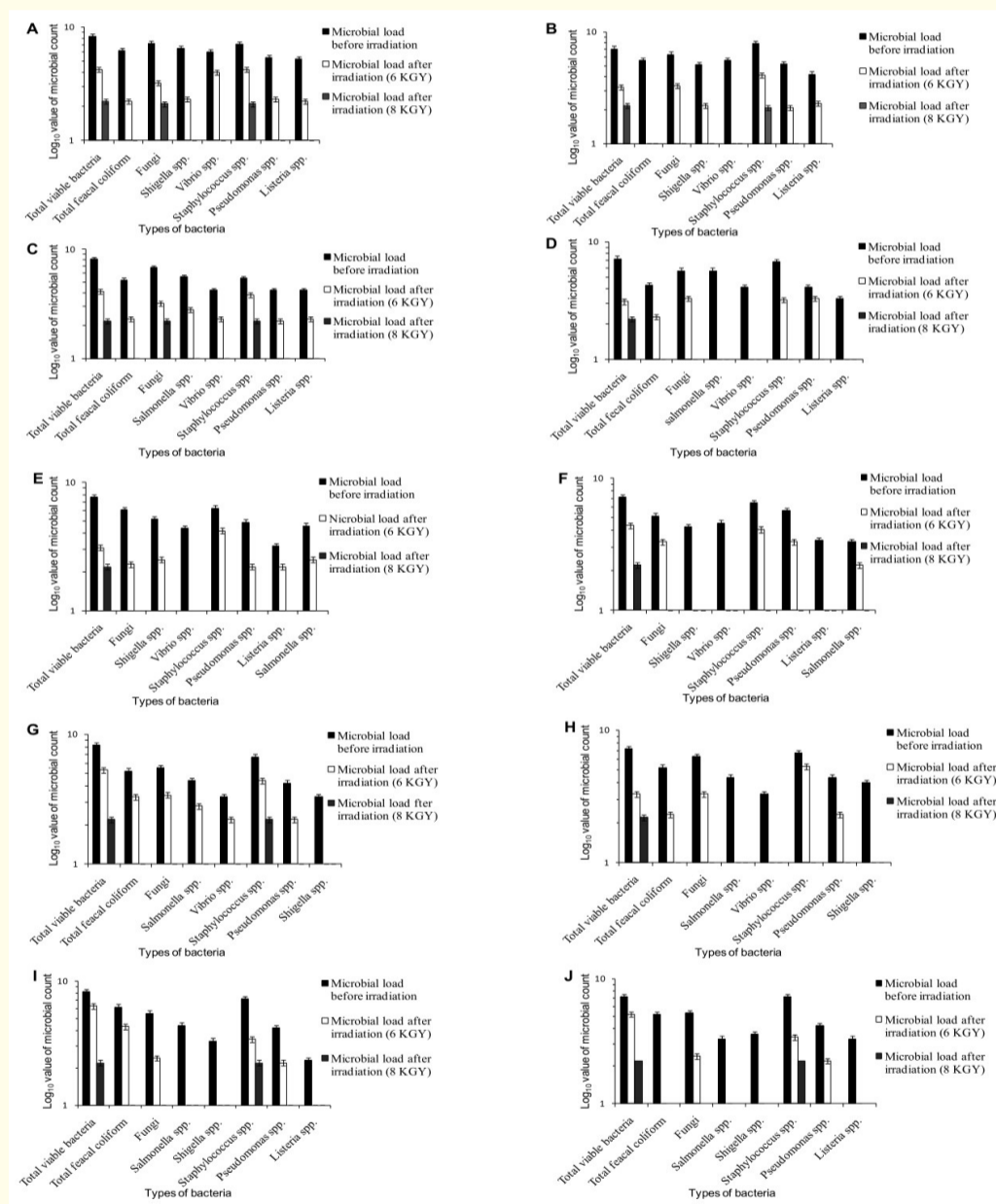
Isolates	TSI			H <sub>2</sub> S reaction	Indole test	MR test	VP test	Citrate Test	Motility test	Oxidase Test
	Slant	Butt	Gas							
<i>Salmonella</i> spp.	R	Y	-	+	-	+	-	-	+	-
<i>Shigella</i> spp.	R	Y	-	-	+/-	+	-	-	-	-
<i>Vibrio</i> spp.	Y	Y	-	-	+	+	-	+	+	+
<i>Staphylococcus</i> spp.	Y	R	+	+	-	+	-	+	+	-
<i>Listeria</i> spp.	Y	Y	-	-	-	+	+	-	+	-
<i>Pseudomonas</i> spp.	R	R	-	-	-	-	-	+	+	+

**Table 1:** Biochemical identification of the pathogenic isolates from sea fish.

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 Chub mackerel, [C-D] for Koral, [E-F] for Hilsha, [G-H] for Shrimp and [I-J] for Red Rupchanda fish samples. A, C, E, G and I: packet washed water samples; B, D, F, H and J: fish blend samples. Brief description of different irradiation doses and the pathogenic profile were stated in Materials and Methods. White bars and light back are pinpointing the irradiated samples (6kGy and 8kGy) respectively while black bars denote the non-irradiated samples.

Descriptively, in case of *Scomber colias*, the fecal count was around  $10^6$  cfu/mL together with the prevalence of *Shigella* spp. up to  $10^6$  cfu/mL, *Vibrio* spp. up to  $10^6$  cfu/mL, *Staphylococcus* spp. up to  $10^7$  cfu/mL, *Pseudomonas* spp. up to  $10^5$  cfu/mL and *Listeria* spp. up to  $10^5$  cfu/mL. *Staphylococcus* spp. was found to be abundant (Figure 1A). Conversely, the fish blend part harbored less microorganisms than the packet washed water (Figure 1A) as the fecal contamination was noticed  $10^5$  up to cfu/mL or cfu/g and others pathogens were also found to be in less quantity than those in the packet washed waters (Figure 1B). In *Lates calcarifer* samples, the packet washed water and fish blend samples were totally free from *Shigella* spp. (Figure 1C and 1D). In both cases (packet washed water and fish blend) the total viable bacteria was found up to  $10^8$  and  $10^7$  cfu/mL or cfu/g, fecally contamination was  $10^5$  and  $10^4$  cfu/mL or cfu/g while fungal contamination was  $10^6$  and  $10^5$  cfu/mL or cfu/g, respectively. However, the Staphylococcal contamination as well as *Listeria* spp. was found to be in higher quantity in fish blend ( $10^6$  cfu/mL or cfu/g) than the packet washed water ( $10^5$  cfu/mL) (Figure 1C and 1D).

In *Tenualosa ilisha* (hilsha), the total viable bacteria and fungi were found in both packet washed water and fish blend within the range of  $10^7$  to  $10^6$  cfu/mL or cfu/g and  $10^7$  to  $10^5$  cfu/mL or cfu/g respectively (Figure 1E and 1F). In *Pandalus borealis* samples, all were found to be free from the contamination of *Listeria* spp. (Figure 1G and 1H). However, *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Pseudomonas* spp. and *Vibrio* spp. were detected within the range of  $10^3$  to  $10^6$  cfu/mL or cfu/g in case of packet washed water and  $10^4$  to  $10^6$  cfu/mL or cfu/g in the fish blend, respectively (Figure 1G and 1H). In *Colossoma macropomum* samples were found to be free of contamination by *Vibrio* spp. (Figure 1I and 1J). However, other microorganisms were present; and like the other samples, the packed washed waters harbored more pathogens than those within the fish blends (Figure 1I and 1J).

Our previous studies revealed that several food born and enteric diseases may be triggered by the huge propagation of resistant bacteria in fish, food and water [3,4,25,36]. Besides, earlier we found a lot of drug-resistant bacteria i.e., *E. coli*, *Salmonella* spp., *Staphylococcus* spp. and *Pseudomonas* spp. within the sea fish samples [4,25]. In the current research using different sea fish samples, *Listeria* spp. has been detected as a fish contaminating agent. The possible sources of such contamination may arise from the beginning of fish after caught, when the sellers use ice for the preservation of fishes. In most of the time the ice is prepared by using contaminated waters. As has been noticed earlier, in the local markets varieties of fishes are kept together and the sellers do not maintain proper aseptic techniques which may creates the possibilities to come in contact of several pathogenic bacteria [8]. Therefore, proper legislative bodies need to be formed with the possible guidelines on aseptic handling and storage of fish for the sake of the consumer safety.

#### Existence of drug-resistant pathogens in fish samples

As discussed in previous studies the drug resistance trait of pathogens proliferated in fish and fish product may trigger serious dilemma in disease medication [4,25,39]. In Bangladesh and in other developing countries, the problem of drug-resistant bacterial proliferation not only within the clinical case but also within food samples including fish is very common which in turn evoke fatality in the mass public health [4,41-44]. Like other food samples, our previous works on sea fish samples revealed the presence of a significant proportion of drug-resistant bacteria [4,25]. In the current study where different sea fishes were microbiologically analyzed, samples were also found to be harbor huge array of resistant strains like *Listeria* spp. showed 100% resistance against AMP, CIP, STE, PEN, TER, CHL and 100% sensitive against CEF, IPM, GEN, AZI, CFX, ERY (Table 2). *Vibrio* spp. exhibited 100% resistance against AMP, STE, PEN, TER and 100% sensitive against CIP, CEF, IPM, GEN, AZI, CFX, ERY, CHL. *Staphylococcus* spp. exhibited 100% resistance against AMP, CIP, STE, PEN, TER and 100% sensitive against AMP, CEF, IPM, GEN, AZI, CFX, ERY, CHL. *Salmonella* spp. was found 100% resistance against AMP, CIP, STE, CEF, PEN and 100% sensitive against IPM, GEN, AZI, TER, CFX, ERY, CHL. *Pseudomonas* spp. showed 100% resistance against AMP, CIP, STE, CEF, IPM, PEN, GEN, AZI, TER and 100% sensitive against ERY, CHL. *Shigella* spp. was found to be resistant against AMP, CIP, STE, PEN, TER and 100% sensitive against CEF, IPM, GEN, AZI, CFX, ERY, CHL (Table 2). Detection of Drug resistant bacteria from such export quality sea fish may also becoming more risky for the global public health as well as a dreadful indication for the local economy [25,45-47].

Before Irradiation												
Isolates Antibiotics	Listeria spp. n = 4		Vibrio spp. n = 4		Staphylococcus spp. n = 5		Salmonella spp. n = 4		Pseudomonas spp. n = 5		Shigella spp. n = 3	
	R	S	R	S	R	S	R	S	R	S	R	S
AMP (10 $\mu$ g)	100%	0%	100%	0%	100%	100%	100%	0%	100%	0%	100%	0%
CIP (5 $\mu$ g)	100%	0%	0%	100%	100%	0%	100%	0%	100%	0%	100%	0%
STE (10 $\mu$ g)	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%
CEF (30 $\mu$ g)	0%	100%	0%	100%	0%	100%	100%	0%	100%	0%	0%	100%
IPM (30 $\mu$ g)	0%	100%	0%	100%	0%	100%	0%	100%	100%	0%	0%	100%
PEN (10 $\mu$ g)	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%
GEN (10 $\mu$ g)	0%	100%	0%	100%	0%	100%	0%	100%	100%	0%	0%	100%
AZI (15 $\mu$ g)	0%	100%	0%	100%	0%	100%	0%	100%	100%	0%	0%	100%
TER (30 $\mu$ g)	100%	0%	100%	0%	100%	0%	0%	100%	100%	0%	100%	0%
CFX (5 $\mu$ g)	0%	100%	0%	100%	0%	100%	0%	100%	0%	0%	0%	100%
ERY (15 $\mu$ g)	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
CHL (10 $\mu$ g)	100%	0%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%

**Table 2:** Antibacterial susceptibility pattern of different pathogenic isolates found in sea fish samples.

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.

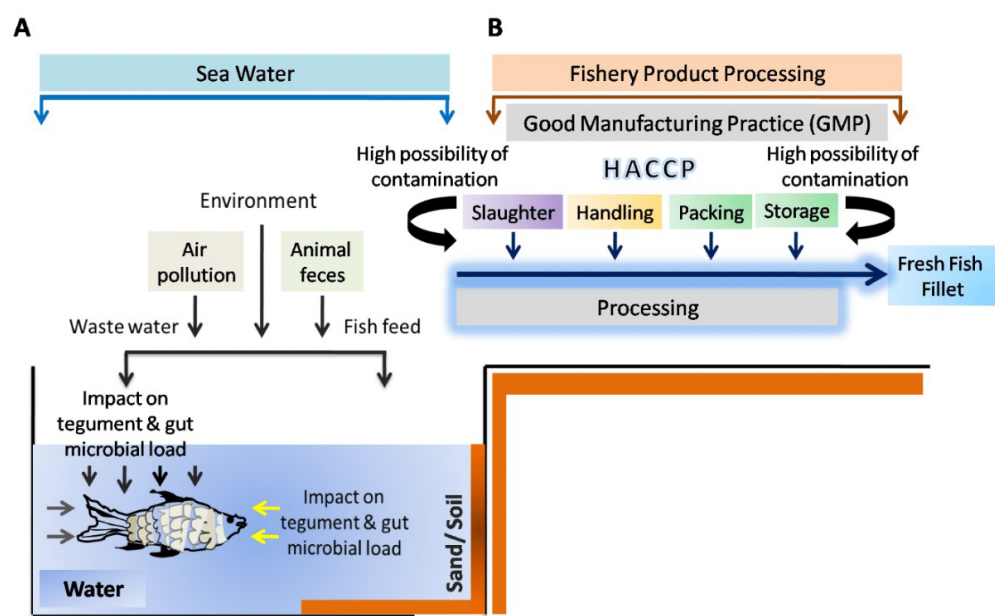
### Effect of $\gamma$ -irradiation (6 kGy and 8 kGy) on the reduction of pathogenic bacteria

Our earlier microbiological survey on *Pampus chinensis* and *Scomberomorus guttatus* revealed the efficacy of  $\gamma$ -irradiation in microbial reduction [4]. Indeed, to enhance the food shelf life and maintaining the proper nutritive value, implementation of the  $\gamma$  -irradiation is significantly effective to reduce the proliferation of bacterial population among the all others methods available [4,25]. As a consequence, present study further extended the arena of investigation by increasing sea fish samples and the radiation doses, and thus attempted to figure out the bactericidal dose of irradiation. As revealed from the current study, after irradiation, the pathogenic load significantly decreased more than 4 log at 6 kGy and interestingly the growth of most pathogens were totally shattered at 8 kGy (Figure 1). In *Scomber colias*, the total viable bacterial load, fecal coliform and fungi from packed washed water were significantly reduced to 4 logs at 6 kGy while the load became absolutely zero at 8 kGy except for the total viable bacteria, fungi and *Staphylococcus* spp. which were noticed to be reduced up to 6 logs from the before irradiation (Figure 1A and 1B). In *Lates calcarifer* samples, in case of both categories (packet washed water and fish blend), the load of all microorganisms including the total viable bacteria, fecal coliform and fungi were found to be reduced within the range of 2 to 4 logs at 6 kGy (Figure 1C and 1D). Certainly, the dose of 8kGy was found to be effective to for a complete reduction all specific pathogens (Figure 1C and 1D). In *Tenualosa ilisha* samples, the reduction rate was calculated for packed washed water up to 4 logs at 6 kGy, and 100% reduction was noticed at 8 kGy except total viable bacteria which was reduced by 5 logs compared to the non-irradiated samples (Figure 1E and 1F). Afterward, in case of fish blend the microbial load was found to be reduced up to 3 logs at 6 kGy and was 100% reduced at 8 kGy (Figure 1E and 1F). In *Pandalus borealis* samples, the reduction rate was calculated for packed washed water up to 3 logs at 6 kGy while 100% reduction in the specific pathogens was noticed at 8 kGy except total viable bacteria which was actually reduced by 6 logs and *Staphylococcus* spp. was reduced by 4 logs (Figure 1G and 1H). In case of fish blend the load was found to be reduced

up 4 logs at 6 kGy and was 100% reduced at 8 kGy (Figure 1G and 1H). However, the growth of *Salmonella* spp. and *Vibrio* spp. was found to be completely diminished by both doses (6 kGy and 8 kGy). In *Colossoma macropomum* samples, the reduction rate was calculated up to 5 logs in both cases (packed washed water and fish blend) at 6 kGy and 100% reduction was noticed at 8 kGy. The count was found to be totally reduced for *Salmonella* spp., *Shigella* spp. and *Listeria* spp. at 6 kGy and 8kGy in both cases (Figure 1I and 1J).

One point needs to be pondered that after irradiation at both 6 kGy and 8 kGy doses, the existence of total viable bacteria, fungi and *Staphylococcus* spp. was noticed in fewer amounts (Figure 1A-1J). Several researches have been approved that among the many conventional techniques, irradiation up to 10 kGy may be more effective and economical against the pathogens which has no harmful effects on consumers health [23]. However, present evaluation successfully provided the experimental evidence that the 8 kGy was effectively reduced the 100% growth of maximum pathogenic bacteria in sea fish samples which would be helpful for the retailer to ensure the good quality and long term sustainability of fish and fish products for the consumers.

Finally, in addition to the irradiation technology to minimize the microbial contamination frequency of sea fish, it's necessary to provide a hygienic environment for fish handling and processing to keep the fishes initially up to the quality both for local consumption and for export purpose. In order to achieve this, research on fish microbiology must concentrate towards the fish habitats which may be influenced by several environmental factors including air and water pollution which in turn negatively influences on healthy growth of the fish habitat. Polluted air consists of harmful gases; i.e., carbon dioxide, methane, etc. which inhibits respiration of fishes. Production of excess amount of toxic oxygen (super radicals, ions, etc.) triggered by the pollution of water causes the death of fishes. Moreover, animal feces containing high amount of toxic nitrogenous compounds may also hamper the health and quality of fishes (Figure 2A). Another aspect of maintaining the fish quality underlies the stages of fish processing, storage and supply within the fisheries industries. Fishes may be contaminated by microorganisms or lose their quality by the fisherman, or by the handler during slaughtering and initial processing and storage. To obtain the best qualified and healthy fish, appropriate rules and regulations should be coordinated by the good manufacturing practices (GMP) and should strictly adhere to the hazard analysis and critical control point (HACCP). Both GMP and HACCP should be implemented by the appropriate authority and personnel during processing, packaging, storage and distribution (Figure 2B). As can be seen from our earlier researches on fish borne microorganisms, another aspect of microbiological infectivity of fish habitat can be triggered by several factors like environment, air, animal feces and waste waters [36,48] which in turn may hinder the normal and healthy growth of sea fish (Figure 2). Therefore, appropriate and continuous mode of remedies to get rid of microbial contamination of fishes urges mostly to maintain healthy food supply for mass people. This will definitely reduce the chances of microbial dissemination within fishes.



**Figure 2:** Schema of fish habitat and fish processing. [A] Within the fish habitat several environmental factors such as air pollution (carbon dioxide, methane, etc.), water pollution (increase in the concentration of super radicals, ions, etc.), animal feces may affect the growth and quality of sea fish by decreasing the level of dissolved oxygen. [B] Processing of sea fish in fisheries industries (slaughtering, handling, storage, distribution, etc.) should be well regulated by good manufacturing practices (GMP) and the hazard analysis and critical control point (HACCP).

## Conclusion

An array of experimental and suggestive data unraveled the fact that different foods including sea fishes may serve as the good reservoir of microbial agents, sometimes the pathogenic activity of those microorganisms may be responsible for food-borne disease outbreaks. The virulence genes of these pathogens may create not only infection of the fish but also become fatal for the consumers. While in Bangladesh the genetic analysis of the export quality shrimp has been performed by our group; however, still the other sea fishes need to be analyzed for the possible expression of the virulent genes. Apart from molecular study, the present research successfully (1) portrayed the pathogenic profile of popular and export quality sea fishes of Bangladesh, (2) figured out the drug-resistance trait of the isolated pathogens which in turn may help the clinical professionals to further work on these isolates to know the molecular mechanism of the drug-resistance gene transmission; and (3) finally the study clearly demonstrated the irradiation dose possessing the bactericidal and fungicidal activity within the fish samples tested. The efficacy of the radiation dose 6 kGy and 8 kGy on microbial reduction is expected to improve the shelf-life as well as the public health of the consumers. Such findings may also help to meet the high export demand of our sea fishes.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

## Bibliography

1. Abbey L., *et al.* "Nutrient content of fish powder from low value fish and fish byproducts". *Food Science and Nutrition* 5.3 (2017): 374-379.
2. Dunlop E., *et al.* "Vitamin D<sub>3</sub> and 25-Hydroxyvitamin D<sub>3</sub> Content of Retail White Fish and Eggs in Australia". *Nutrients* 9.7 (2017): 647.
3. Hassan MR., *et al.* "Microbiological study of sea fish samples collected from local markets in Dhaka city". *International Food Research Journal* 20.3 (2013): 1491-1495.
4. Noor R., *et al.* "Microbiological analysis of major sea fish collected from local markets in Dhaka city, Bangladesh". *Journal of Microbiology Biotechnology and Food Science* 2.4 (2013): 2420-2430.
5. Noor R., *et al.* "Molecular characterization of the virulent microorganisms along with their drug resistance traits associated with the export quality frozen shrimps in Bangladesh". *Springer Plus* 3 (2014): 469.
6. Noor R., *et al.* "Demonstration of virulent genes within *Listeria* and *Klebsiella* isolates contaminating the export quality frozen shrimps". *International Aquatic Research* 7.2 (2015): 157-161.
7. DoF. "Fishery Statistical Yearbook of Bangladesh 2004-2005". Department of Fisheries, Ministry of Fisheries and Livestock, Dhaka (2006).
8. Sultana S., *et al.* "Microbiological quality analysis of shrimps collected from local market around Dhaka city". *International Food Research Journal* 21.1 (2014): 33-38.
9. Eze EI., *et al.* "Isolation and identification of pathogenic bacteria associated with frozen mackerel fish (*Scomber scombrus*) in a humid tropical environment". *African Journal of Agriculture Research* 6.8 (2011): 1918-1922.



10. Rathlavath S, *et al.* "Comparative isolation and genetic diversity of *Arcobacter* sp. from fish and the coastal environment". *Letter of Applied Microbiology* 65.1 (2017): 42-49.
11. Lee RJ, *et al.* "Bacterial pathogens in seafood". In T Borresen, Improving seafood products for the consumer. Woodhead Publishing series in food science, technology and nutrition, Cambridge: Woodhead Publishing Limited (2008): 1-70.
12. Lazado CC, *et al.* "Prospects of host-associated microorganisms in fish and penaeids as probiotics with immunomodulatory functions". *Fish Shellfish Immunology* 45.1 (2015): 2-12.
13. Moini S, *et al.* "Effect of Gamma Radiation on the Quality and Shelf Life of Refrigerated Rainbow trout (*Oncorhynchus mykiss*) fillets". *Journal of Food Protection* 72.7 (2009):1419-1426.
14. Rostamzad H, *et al.* "Inhibitory impacts of natural antioxidants (ascorbic and citric acid) and vacuum packaging on lipid oxidation in frozen Persian Sturgeon fillet". *International Iranian Journal of Fisheries Science* 9.2 (2010): 279-292.
15. Casti D, *et al.* "Occurrence of Nematodes of the Genus *Anisakis* in Mediterranean and Atlantic Fish Marketed in Sardinia". *Italian Journal of Food Safety* 6.1 (2017): 6185.
16. Neela FA, *et al.* "Occurrence of antibiotic resistant bacteria in pond water associated with integrated poultry-fish farming in Bangladesh". *Sains Malaysiana* 44.3 (2015): 371-377.
17. Carlson JM, *et al.* "Microbiome disruption and recovery in the fish *Gambusia affinis* following exposure to broad-spectrum antibiotic". *Infection and Drug Resistance* 10 (2017): 143-154.
18. Songe MM, *et al.* "Antimicrobial Resistant *Enteropathogenic Escherichia coli* and *Salmonella* spp. In Houseflies Infesting Fish in Food Markets in Zambia". *International Journal of Environmental Research and Public Health* 14.1 (2017): 21.
19. Gelman A, *et al.* "Effects of storage temperature and preservative treatment on shelf life of the pond-raised freshwater fish, silver perch (*Bidyanus bidyanus*)". *Journal Food Protect* 64.10 (2001): 1584-1591.
20. Haghparast S, *et al.* "Antioxidant properties of sodium acetate, sodium citrate and sodium lactate on lipid oxidation in rainbow trout (*Onchorhynchus mykiss*) sticks during refrigerated storage (4°C)". *International Indian Journal of Fisheries Science* 9.1 (2010): 73-86.
21. Motalebi AA, *et al.* "Impact of whey protein edible coating on chemical and microbial factors of gutted tilapia during frozen storage". *International Iranian Journal of Fisheries Science* 9.2 (2010): 255-264.
22. Saritha K, *et al.* "Physico chemical and sensorial characteristics of commercial seafood pickles Tuticorin super market, Tamil Nadu, India". *International Food Research Journal* 21.2 (2014): 649-654.
23. WHO. "Wholesomeness of irradiated foods, Technical Report Series 659". Geneva, World Health Organization (1981).
24. Chakraborty S, *et al.* "Effect of gamma radiation on the sensory, chemical and microbiological changes in two strains of climbing perch. *Anabas, testudineus*, Bloch, 1792". *Journal of the Asiatic Society of Bangladesh*, Science 38.2 (2012): 183-188.
25. Acharjee M, *et al.* "Validation of  $\gamma$ -irradiation in controlling microorganisms in fish". *Nutrition and Food Science* 44 (2014): 258-266.
26. Ehlermann DAE. "Wholesomeness of Irradiated Food". *Radiation Physics and Chemistry* 129 (2016): 24-29.
27. El-Ghafour S and Zakar AH. "Impact of Gamma Irradiation on the Quality of Tilapia Fish (*Oreochromis niloticus*) Fillets Stored under Refrigerated Condition". *International Journal of ChemoTech Research* 10.2 (2017): 573-581.

28. APHA (American Public Health Association). Standard Methods for the Examination of Water and Wastewater. 20<sup>th</sup> Edition, American Public Health Association, Washington, D.C (1998).
29. Cappuccino JG and Sherman N. "Microbiology-A Laboratory Manual". The Benjamin/Cummings Publishing Co, Inc, California (1996).
30. Colwell RR. "Bacterial Death Revisited, in Non-culturable Microorganisms in the Environment". (Eds.: R.R. Colwell, D.J. Grimes). ASM, Washington DC USA. (2000): 325-342.
31. Rahman F and Noor R. "Prevalence of pathogenic bacteria in common salad vegetables of Dhaka Metropolis". *Bangladesh Journal of Botany* 41.2 (2012): 159-162.
32. Ramamurthy T, *et al.* "Current Perspectives on Viable but Non-Culturable (VBNC) Pathogenic Bacteria". *Frontiers in Public Health* 2 (2014): 103.
33. Tortora GJ, *et al.* "Microbiology: An Introduction Benjamin Cummings". 10<sup>th</sup> edition, Benjamin Cummings, USA (2008).
34. Oliver JD. "Recent Findings on the Viable but Nonculturable State in Pathogenic Bacteria". *FEMS Microbiology* 34.4 (2010): 415-425.
35. Zhao X, *et al.* "Current Perspectives on Viable but Non-culturable State in Foodborne Pathogens". *Frontiers in Microbiology* 8 (2017): 580.
36. Acharjee M, *et al.* "Bacterial proliferation in municipal water supplied in Mirpur locality of Dhaka city, Bangladesh". *CLEAN- Soil Air Water* 42 (2013): 434-441.
37. Bauer AW, *et al.* "Antibiotic susceptibility testing by a standardized single disc method". *American Journal of Clinical Pathology* 45.4 (1966): 493-496.
38. Ferraro MJ, *et al.* "Performance standards for antimicrobial Susceptibility testing". NCCLS, Pennsylvania, USA (2001).
39. Dutta S, *et al.* "Study of antimicrobial susceptibility of clinically significant microorganisms isolated from selected areas of Dhaka, Bangladesh". *Bangladesh Journal of Medical Science* 12.1 (2013): 34-42.
40. Hasan R, *et al.* "Prevalence of Vancomycin Resistant *Staphylococcus aureus* (VRSA) in Methicillin Resistant *S. aureus* (MRSA) Strains Isolated from Burn Wound Infections". *Tzu Chi Medical Journal* 28.2 (2016): 49-53.
41. Dib AL, *et al.* "Prevalence of microbial contamination of fresh seafood product sold in Constantine, Algeria". *Environmental Skeptics and Critics* 3 (2014): 83-87.
42. Chowdhury FFK, *et al.* "Maintenance of Environmental Sustainability Through Microbiological Study of Pharmaceutical Solid Wastes". *CLEAN - Soil, Air, Water* 44.3 (2015): 309-316.
43. Obaidat MM and Bani-Salman AE. "Antimicrobial Resistance Percentages of *Salmonella* and *Shigella* in Seafood Imported to Jordan: Higher Percentages and More Diverse Profiles in *Shigella*". *Journal of Food Protection* 80.3 (2017): 414-419.
44. Wieczorek K and Osek J. "Prevalence genetic diversity and antimicrobial resistance of *Listeria monocytogenes* isolated from fresh and smoked fish in Poland". *Food Microbiology* 64 (2017): 164-171.
45. Bennett PM. "Plasmid encoded antibiotic resistance: Acquisition and transfer of antibiotic resistance genes in bacteria". *British Journal of Pharmacy* 153.1 (2008): S347-S357.
46. Canton R. "Antibiotic resistance genes from the environment: A perspective through newly identified antibiotic resistance mechanisms in clinical setting". *European Society of Clinical Microbiology and Infectious Diseases* 15.1 (2009): 20-25.

47. Hung DT and Kaufman BB. "The Fast Track to Multidrug Resistance". *Molecular Cell Biology* 37.3 (2010): 297-298.
48. Munshi SK, *et al.* "Detection of virulence potential of diarrheagenic *Escherichia coli* isolated from surface water of rivers surrounding Dhaka city". *Journal of Bangladesh Academy of Science* 36.1 (2012): 109-121.

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