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REGULAR ARTICLE

MICROBIOLOGICAL STUDY OF MAJOR SEA FISH AVAILABLE IN LOCAL MARKETS OF DHAKA CITY, BANGLADESH

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ABSTRACT

Present study attempted to determine the prevalence of pathogenic microflora along the two major sea fish samples: Rupchanda (*Pampus chinensis*) and Surmai (*Scomberomorus guttatus*), collected from local market in Dhaka city. Most of the fishes collected were found to be contaminated with a huge number of pathogens within a range of 2.0×10^{2}-1.9×10^{9} cfu.mL^{-1} or cfu.G^{-1}. The study of antibiogram showed a number of isolates to be drug-resistant. The investigation also endeavored to evaluate the efficiency of gamma (γ) irradiation on the pathogenic reduction besides the traditional means. The pathogenic load was found to be reduced by an irradiation dosage of 3 kilo Gray (kGy).

Keywords: Sea fish, pathogens, antibiotic resistance, irradiation, microbiological quality

INTRODUCTION

Fish and fish products are one of the major food components of humans and animals from the ancient period. In Bangladesh, around 60% of animal protein is supplied from fish, and 8% of total export earnings come from fish and fish products. Unfortunately, a large amount of fish spoils every year in the country due to the growth and activity of pathogenic microorganisms. Export market of Bangladesh is threatened with low quality processed foods which may be contaminated with pathogenic bacteria such as coliform, faecal coliform, *Listeria monocytogenes*, *Clostridium botulinum*, *Vibrio cholerae*, *Shigella* spp., *Salmonella* spp., streptococci and *Staphylococcus aureus*. Such pathogenic flora of living fish depends on the microbial content of the aquatic habitat (**Frazier and Westhoff, 1995; Eze *et al*., 2011)**. Together with causing an array of diseases upon consumption of microbiologically spoiled fish, the off-odor and off-taste of the products caused by oxidation of lipids and some other metabolites may largely affect the consumer acceptability (**Moini *et al*, 2009; Rostamzad *et al*., 2010**). Rupchanda is considered as the most popular and costly sea fish in Bangladesh. Both dried and fresh forms of this fish have a huge demand among the consumer levels including the tourists. Mackerel, locally known as Surmai, is another major sea fish in the country which is not only consumed in the cooked form, but also is used to make fish pickle. Assurance of both nutritional and microbiological quality of these fishes thus claims significance. However, no pathogenic surveillance on such sea fishes has been done so far in Bangladesh.

An important aspect is the successful preservation of fish and the subsequent abolition of pathogens. Besides the traditional methods such as ice storage, rapid chilling, freezing, smoking and heating (**Himelbloom *et al*., 1994**), the use of organic acids, antimicrobials (**Gelman *et al*., 2001**), antioxidants (**Haghparast *et al*., 2010**), edible coating (**Motalebi *et al*., 2010**), modified atmosphere packaging (**Masniyom *et al*., 2002**) and ionizing radiation (**Savvaidis *et al*., 2002; Chouliara *et al*., 2004; Erkan and Ozden, 2007**) are also well known methods applicable for the elimination of pathogens. However, the major problem of distribution of seafoods including Rupchanda and Surmai fishes is their susceptibility to microbial spoilage (**Ozden and Erkan, 2010**). Therefore, it is crucial to estimate the rate of spoilage and the preventive strategy of the microbiological spoilage of fish of interest to ensure food safety.

Another important consideration is to be given to the antibiotic resistance trait of the pathogens colonizing the fish, since the trait significantly hinders the disease medication

(**Tenover, 2006**). The extensive misuse of antibiotics led to the development of serious problems of resistance and hence limits the usefulness of antibiotics to eliminate bacterial infections (**Mathew *et al*., 2007; Allerberger and Mittermayer, 2008**). In Bangladesh, a study showed that more than 70% infecting pathogens were resistant against at least one of the commonly used antibiotics (**Jilani *et al*., 2008**). A habitual conductance of antibiogram thus claims its importance from the view of public health importance.

Along these lines, present study examined for the first time in Bangladesh the pathogenic prevalence among the Rupchanda and Surmai fishes, conducted the study of the antibiotic resistance patterns of the pathogenic isolates, and finally attempted to evaluate the efficacy of ionizing radiation (γ -irradiation) on the reduction of those pathogens.

MATERIAL AND METHODS

Study area and sampling

Twelve Rupchanda and 12 Surmai fish samples were collected randomly from different locations in Karwan Bazar fish market within a time frame of February, 2012 to July, 2012. Samples were collected aseptically early in the morning and taken in sterile polyethylene bags with ice and transported immediately to the laboratory.

Sample processing, irradiation and microbiological analysis

The length and weight of the samples were recorded and then each sample was cut into 2 pieces and washed with distilled water. Each piece of samples were put in separate polythene packets (sterilized by irradiation at 15 kGy), and sealed properly. Half of the samples were irradiated at 3 kGy using 60 cobalt radiation source (provided by Board of Radiation and Isotope Technology, India) for 30 minutes, and the remaining half were subjected to pathogenic study (i.e., non-irradiated). Both fresh (non-irradiated) and irradiated samples, packets and fishes were washed with peptone buffer water and each piece of fishes was homogenized with normal saline. Then the packet- and fish washed water, and the fish blend samples were serially diluted up to 10^{-5}.

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

The 0.1 ml of suspension from each dilution of the samples was spread onto Nutrient agar, Membrane Fecal Coliform (mFC) agar, Sabouraud Dextrose Agar (SDA) and Manitol Salt Agar (MSA) plates for the estimation of TVB, TFC, Fungal count and *Staphylococcus aureus*, consecutively. For TVB and Staphylococcal assay, plates were incubated at 37 ^{o}C for 24 hours while for estimating the fecal coliforms, plates were incubated at 44.5 ^{o}C for 24 hours. For fungal assay, SDA plates were incubated at 25 ^{o}C for 48 hours.

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

The 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. However, the absence of growth was assumptive of microbial cells of being in stressed condition or in viable but nonculturable (VBNC) state (**Colwell, 2000; Oliver, 2005**). As a result, enrichment was performed for *V. cholerae* in alkaline peptone water (APW), and *Salmonella* and *Shigella* spp. in the selenite cystine broth. For the isolation of *Listeria* spp., 0.1 ml of suspension was spread onto *Listeria* identification media and plates were incubated at 37 ^{o}C for 24 hours. Finally, the standard biochemical tests were performed to confirm all the isolates (**Cappuccino and Sherman, 1996**).

Statistical analysis

All the experiments were performed in triplicate. Statistical analyses were performed by determining the p-value through *t* test. Errors were also calculated.

Determination of antimicrobial susceptibility

Isolates were examined for antibiotic susceptibility by disc diffusion assay on Mueller-Hinton agar (Difco, Detroit, MI) against ampicillin 10 μg.disk^{-1}, amoxicillin 10 μg.disk^{-1}, ciprofoxacin 5 μg.disk^{-1}, ceftriazone 30 μg.disk^{-1}, nalidixic acid 30 μg.disk^{-1}, imipenem 30 μg.disk^{-1}, chloramphenicol 10 μg.disk^{-1}, trimethoprime-sulfomethoxazole 25 μg.disk^{-1}, gentamycin 10 μg.disk^{-1}, piperacillin 10 μg.disk^{-1} (**Bauer *et al*., 1966; Ferraro, 2001**).

RESULTS AND DISCUSSION

Present study showed an inclusive report on the pathogenic proliferation and their drug resistance pattern among the major sea fishes not only of local importance but are also significant in a broader aspect of global consumption when exported.

Prevalence of pathogenic microorganisms

All the samples exhibited higher pathogenic loads which were biochemically identified. In case of Rupchanda fish, the total viable bacteria for packet washed water was found to be 1.4×10^{8} cfu.mL^{-1}. The load was 1.5×10^{8} cfu.mL^{-1} and 2.5×10^{6} cfu.G^{-1} for fish washed water and fish blend samples, respectively. The prevalence of pathogenic bacteria in packed washed water was higher in case of *Listeria* spp. detected as 7.3×10^{5} cfu.mL^{-1} followed by *Vibrio* spp. (6.3×10^{5} cfu.mL^{-1}), *Staphylococcus* spp. (4.2×10^{6} cfu.mL^{-1}) and *Shigella* spp. (1.5×10^{4} cfu.mL^{-1}), consecutively.

Most of the fish washed and fish blend samples were contaminated with *Shigella* spp. (1.4×10^{3} cfu.mL^{-1} and 3.2×10^{5} cfu.G^{-1}, respectively), *Listeria* spp. (5.1×10^{5} cfu.mL^{-1} and 4.2×10^{4} cfu.G^{-1}, respectively) and *Staphylococcus aureus* (6.8×10^{7} cfu.mL^{-1} and 4.1×10^{7} cfu.G^{-1}, respectively). No *Vibrio* spp. was detected in the fish blend samples but in the fish washed water, the load was found to be 4.2×10^{5} cfu.mL^{-1}. *Salmonella* spp. was absent in all categories of samples. Except fish blend samples, fecal coliforms were detected in packet washed and fish washed water samples only. Fungal growth was observed in all samples ranging from 1.1×10^{4} to 3.7×10^{6} cfu.mL^{-1} or cfu.G^{-1}.

In Surmai fish samples, the total viable bacteria were found in the range of 1.1×10^{6} - 1.7×10^{9} cfu.mL^{-1} or cfu.G^{-1}. No fecal coliform and *Shigella* spp. was found. However, *Salmonella* spp. was present in fish washed water (1.9×10^{5} cfu.mL^{-1}) and fish blend (3.3×10^{5} cfu.G^{-1}) samples. Other pathogens including *Vibrio* spp., *Listeria* spp, *Staphylococcus* spp. and fungi were present in all categories of sample within a range of 4.0×10^{4} - 6.5×10^{5} cfu.mL^{-1} or cfu.G^{-1}, 1.0×10^{5} - 4.6×10^{5} cfu.mL^{-1} or cfu.G^{-1}, 2.2×10^{5} - 4.7×10^{6} cfu.mL^{-1} or cfu.G^{-1} and 1.1×10^{4} - 3.6×10^{5} cfu.mL^{-1} or cfu.G^{-1}, consecutively.

Overall, the samples studied in the present investigation exhibited a huge array of bacterial and fungal pathogens, posing a substantial risk on the public health. The possible sources of such contamination might underlie the following facts: 1) in local markets, different kinds of fishes are kept together and the sellers do not maintain proper hygiene

which creates the possibilities to come in contact of several pathogens; 2) moreover, the sellers use ice, mostly prepared using contaminated water, to preserve fish after being caught which may be a potential source of contamination. Therefore, aseptic handling, proper storage condition and maintaining homeostatsis during lag between fishing and marketing claim an appropriate practice of hygeine for ensuring the microbiological quality of fish as well the consumer safety.

It is worth to note that the pathogens existing in the contaminated foods may harbor virulence genes which might be responsible for disease outbreaks (**Gubala and Proll, 2006; Bhatta *et al*., 2007; Jakee *et al*., 2009**). Detection of such virulence genes in the fish samples would further unveil the overall quality of fish products.

Effect of γ-irradiation on the pathogenic reduction

Another important consideration during export of the fish of interest is to extend the shelf life. In the current study, γ-irradiation was employed in laboratory scale for an enhanced shelf-life of the fish samples as well as to evaluate the inhibitory effect on the pathogens. A dose of 3 kGy was applied based on the fact that 1.5-3 kGy can effectively reduce the load of spoilage microorganisms under refrigeration and thereby improve the shelf-life of seafoods. After irradiation, the pathogenic load was found to be significantly decreased (Figure 1).

In Rupchanda fish samples, the total viable bacterial count was reduced by two logs in all categories of samples (Figure 1A, B & C). Interestingly, in all three categories of samples, the fecal coliform, *Vibrio* spp. and *Listeria* spp. reduced to nil. The other pathogens found to be 100% reduced in fish blend and fish washed water except *Staphylococcus* spp. which exhibited reduction by two logs in fish washed water samples (Figure 1B & C). In packet washed water, a significant reduction by three logs was observed for *staphylococcus* spp., whereas fungi and *Shigella* spp. was reduced by two and one log, respectively (Figure 1A).

In Surmai fish samples, two log reductions was observed for the total viable bacteria in all category of samples and for *Staphylococcus* spp. in packet washed water and fish washed water samples (Figure 1D, E & F). A notable reduction by two logs was also found for *Salmonella* spp. in fish washed water, for *Listeria* spp. in fish washed water and fish blend samples and for *Vibrio* spp. in fish blend samples (Figure 1E & F). The load of fungi was reduced by one log in fish washed water but not significantly reduced in packet fish water. The pathogens were found to be reduced to nil in all other cases (Figure 1D, E & F).

Existence of drug-resistant pathogens in fish samples

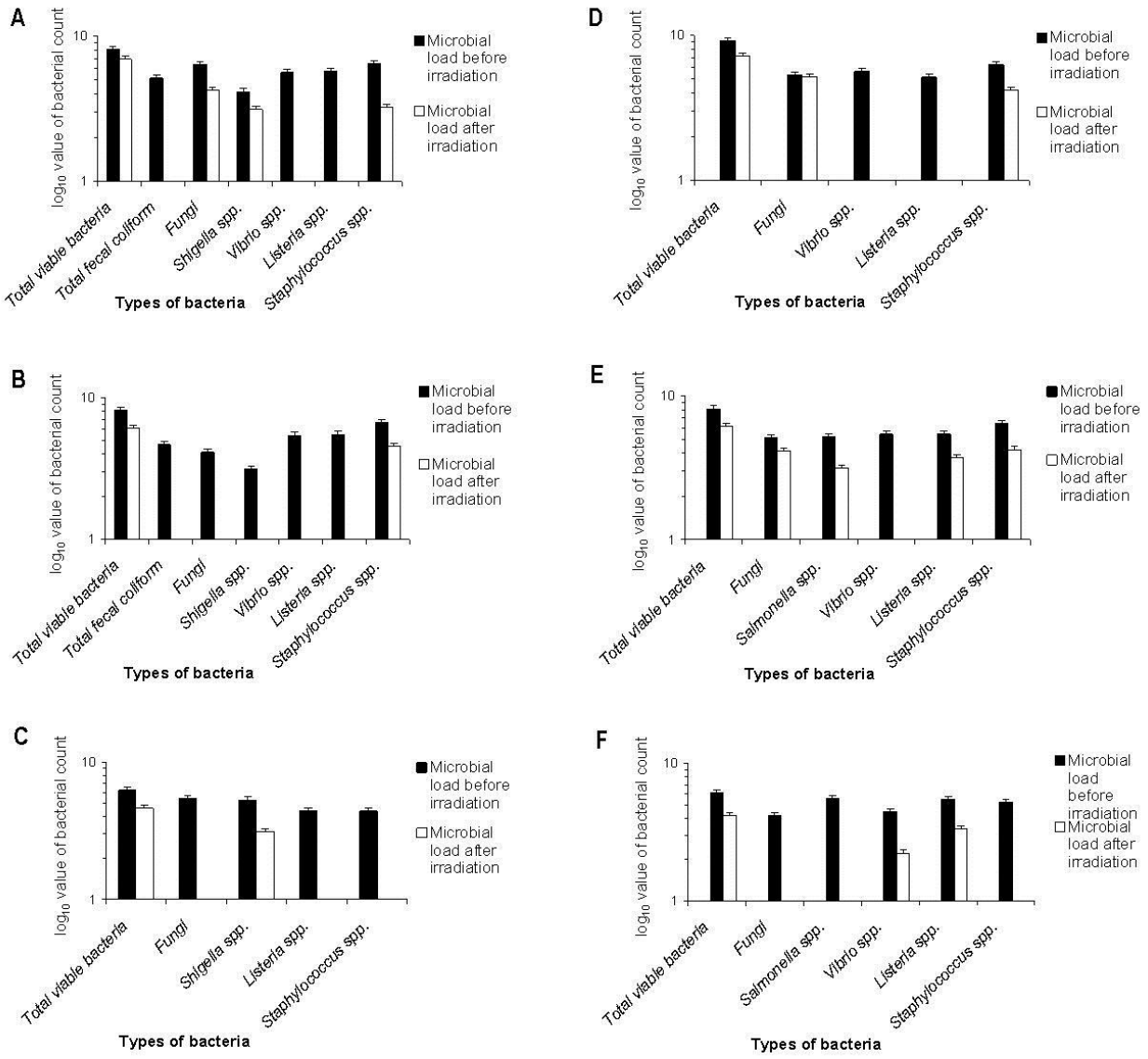

Figure 1 Effect of □-irradiation on the reduction of microbial pathogenic load in Rupchanda (A-C) and Surmai (D-F) fish samples. A, D: packet washed water samples; B, E: fish washed water samples; and C, F: fish blend samples. Irradiation and the pathogenic analysis were carried out as stated in Materials and Methods. White bars are indicative of irradiated samples while black bars denote the non-irradiated samples

Most of the pathogens isolated during the current investigation were found to be resistant against commonly used antibiotics including ampicillin, ciprofloxacin, amoxicillin, chloramphenicol, trimethoprime-sulfomethoxazole, while sensitive to imipenem, piperaciline, nalidixic acid, gentamycin and ceftriazone (Table 1). The drug-resistance might be due to several mechanistic, epidemiologic and genetic factors (**Bennett, 2008; Canton, 2009; Hung and Kaufman, 2010**). Resolving such drug-resistance would be important enough to the effective medication during fish borne disease outbreaks.

Table 1 Antibiogram of the pathogenic isolates

	Pathogens									
	Shigella spp. n=12		*Salmonella* spp. n=8		*Vibrio* spp. n=21		*Listeria* spp. n=24		*Staphylo-coccus* spp. n=24	
Antibiotics	R	S	R	S	R	S	R	S	R	S
AMP	69%	31%	75%	25%	80%	20%	99%	1%	90%	10%
CIP	10%	90%	80%	20%	10%	90%	68%	32%	ND	ND
PIP	ND	ND	ND	ND	ND	ND	100%	0%	90%	10%
CEF	12%	88%	30%	70%	70%	30%	ND	ND	ND	ND
AMO	27%	73%	10%	90%	ND	ND	90%	10%	80%	20%
IPM	10%	90%	15%	85%	70%	30%	ND	ND	ND	ND
CHL	58%	42%	40%	60%	30%	70%	35%	65%	ND	ND
TMP-SUL	12%	88%	15%	85%	70%	30%	78%	22%	30%	70%
GEN	0%	100%	30%	70%	ND	ND	15%	85%	35%	75%
NALI	100%	0%	10%	90%	85%	15%	ND	ND	ND	ND

Legend: ND − Not done, N − Number of isolates, R − Antibiotic Resistant, S − Antibiotic Sensitive, AMP − Ampicillin 10µg, AMO − Amoxicillin 10µg, CIP − Ciprofloxacin 5µg, CEF − Ceftriazone 30µg, NALI − Nalidixic Acid 30µg, IPM − Imipenem 30µg, CHL − Chloramphenicol 10µg, TMP/SUL − Trimethoprime-sulfomethoxazole 25µg, GEN − Gentamycin 10µg, PIP − Piperaciline 10µg.

CONCLUSION

Present study portrayed for the first time in Bangladesh a complete pathogenic profile of the two most popular sea fishes: Rupchanda and Surmai, which are imperative not only for consumption by local people but also possessing high export demand. The prevalence of pathogenic bacteria and fungi in the studied fish samples envisages the significant public health risk. Besides, the drug resistance traits of the pathogens projected from the investigation could largely aid in the appropriate treatment during fish borne diseases

outbreaks. Subsequently, the proposed irradiation method could also be in use for the export quality Ruchanda and Surmai fishes. The findings of the present study could be applied for the improvement of shelf-life of the export quality Rupchanda and Surmai fishes and would suggest a guideline for maintaining fish quality in resource poor settings where advanced molecular studies including the toxigenic- or antibiotic resistance gene detection is not possible.

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