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Comparative microbiological analysis of raw fishes and sun-dried fishes collected from the Kawran bazaar in Dhaka city, Bangladesh

[1]Nur, I.T., [1]Ghosh, B.K. and [2,*]Acharjee, M.

[1]*Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka-1217, Bangladesh*

[2]*Department of Molecular and Developmental Biology, Graduate School of Science and Technology, Shizuoka University, Oya 836, Suruga-ku, Shizuoka, 422-8529, Japan*

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Abstract

Along with the raw fishes, dry fishes also have a huge contribution to meet up the demand of protein in our daily meal. The assay of microbiological quality is therefore needed to ensure the public health safety. The present study was emphasized on the existence of pathogenic bacteria in raw and dry fish. A total of 50 samples of raw fishes and sun-dried fishes was accumulated aseptically for microbiological quality analysis. Isolation of bacteria was done by spread plate method. All the samples including both (raw and dry) fishes harbored bacteria and fungi up to 10^6 CFU/g. *E. coli* was found in all samples as a specific pathogen. In case of raw fishes total viable count (TVC) and total coliform count (*E. coli*) were recorded up to $2.5x10^6$ CFU/g and $5.2 x10^4$ CFU/g respectively whereas a significant load of *Salmonella* spp. was observed in almost all samples. *Staphylococcus* spp. and *Pseudomonas* spp. were present up to $5 x10^2$ CFU/g and $1.8 x 10^2$ CFU/g respectively. Likewise, total viable count (TVC), total coliform count (*E. coli)* and fungal load were recorded in dry fish up $3.50 x 10^5$ CFU/g, $1.2 x10^3$ CFU/g respectively. Fungal growth was observed in all experimental raw and dried fishes. For most of the pathogenic isolates, higher rates of resistance were found against Ceftriaxone, Penicillin, Nalidixic acid, Neomycin. On the other hand, most of the isolates were found to retain higher sensitivity against Imipenem, Ciprofloxacin, Tetracyclin and Amoxicillin. This data suggested that the dry fish harbored fewer bacteria than raw fish and sun drying method is still a useful technique for the preservation of fish.

1. Introduction

Fishes play an important role in human diets as good sources of animal protein that also provide other important elements necessary for the maintenance of healthy bodies (Dewi *et al*, 2011; Ravichandran *et al*., 2012). Because of its high nutritive value, sea fishes are a major vehicle for pathogenic microorganisms (Das *et al*., 2007; Geetha *et al*., 2014). Fishes begin hostile by both the aquatic environment and post-harvesting condition (Al-Sheraa *et al*., 2018). Due to the attack of pathogenic bacteria and fungi a wide range of fish spoilage occurs which adversely affect the economic condition in Bangladesh and as well as the public health safety (Khan and Khan, 2001; Musa *et al.,* 2010; Dewi *et al*., 2011). Consumption of improperly cooked fish may sometimes cause fish-bearing intoxication, which is due to continual subjection of the fish to the microbes present in the water or during transportation (Shabeeb *et al*., 2016).

Several methods have been implementing over the year in the world for preserving fish to extend its shelf life including drying, salting and smoking. Sun drying of fishes is an efficacious and formerly known method of fish preservation. In tropical countries like Bangladesh, fish drying is a prime and inexpensive preservation method (Balachandran, 2001). Dried fish known as 'Shutki' in Bangladesh is one of the popular food items to the various consumers in the globe due to its high protein content and other essential nutrients as well (Arannilewa *et al*., 2006). In Bangladesh point of view, the fish drying process is widely performed by the community of the coastal region (Balachandran, 2001). Fish drying process preserves the quality for a prolonged time and offers a unique taste, flavored and minimum deterioration in the product. Traditional drying methods cannot be applied during the monsoon because of the high humidity. By this time, the fish can absorb the moisture and microbial population such as bacteria, fungi and even viruses (Chowdhury and Bagluis, 1997; Khan

*Corresponding author.
Email: *mrityunjoy_111@yahoo.com*

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and Khan 2001; Azam, 2002). Fungal contamination is a common problem and adversely affects the quality of fishes. The presence of different types of fungi in dried fishes has been reported earlier (FAO 1982; Gupta and Samuvel 1985; Atapattu and Sararajeewa, 1990; Prakash, 2011; Shanthini and Patterson, 2012).

However, the main principle of fish drying is to stop the progress of muscle enzymatic activity and microbial growth by reducing the water activity of fish. Other problems markedly evident with dried fish are the indiscriminate use of various types of pesticides (Nowsad, 2005). In some of the cases, due to the chemical agent histamine, the food borne illness such as scombroid poisoning is observed in dry fishes (Patterson and Ranjitha, 2009). In sun-dried fishes, *E. coli* is responsible for the production of histamine. Infrequently, *Salmonella* and *Staphylococcus* species also produce histamine residue (Kim *et al*., 2003). Currently, the demand of drying fish is decreasing in the market due to the anomaly at different stages of dry fish processing including low-quality raw fish for drying, traditional drying practices, unhygienic and improper sanitation facilities and random use of unaccredited chemicals and insecticides (Yam *et al.,* 2015; Hasan *et al.,* 2016). A few works have been done on the quality assessment of dried fish in our country. Considering all these facts, the present study was designated to determine and compare the microbiological quality and the drug resistant feature of the bacterial species among raw fishes and sun-dried fishes.

2. Materials and methods

2.1 Study area and sample collection

The experiment was executed with a total 50 samples on both raw fish (five samples of each raw fish type, n=25) and dry fish (five samples of each dry fish type, n=25) of the same species including Rupchada (Pomfret; *Brama brama*), Lote (Bombay duck; *Harpadon neherreus*), Chingri (Prawn; *Penaeus monodon*), Puti (Swamp barb; *Puntius chola*), Mola carplet (*Amblyphryngodon microlepi*). The samples were collected from Kawran bazar in Dhaka city using a sterile aseptic container together with ice for raw fishes. A total of 20 g of each the raw and dried of each fish was homogenized with 180 g of sterile normal saline. The homogenized suspension was subjected to serial dilutions (10-fold) up to 10^{-4} with normal saline (Nur *et al*., 2020).

2.2 Enumeration of total viable bacteria and fungus

A total of 0.1 mL of each sample was spread onto nutrient agar and Sabouraud dextrose agar (SDA) for enumerating total viable bacteria (TVB) and total fungal respectively. For TVB, plates were incubated at 37^{o}C. For fungal assay, plates were incubated at 25^{o}C for 3 days (Acharjee *et al*., 2014).

2.3 Isolation of total coliform and fecal coliform

For enumeration of coliforms and fecal coliforms MacConkey agar and membrane fecal coliform agar (mFC) are used respectively. A total of 0.1 mL suspension was spread over MacConkey agar and mFC agar. For the isolation of *Escherichia coli* and *Klebsiella* spp., plates were incubated at 37^{o}C for 18-24 hrs. Presence of *E. coli* was further confirmed by the appearance of bluish-black colonies with green metallic sheen on eosin-methylene blue (EMB) agar (Acharjee et al., 2014). On the other hand, while for fecal coliforms, plates were incubated at 44.5^{o}C for 24 hrs.

2.4 Isolation of other pathogenic bacteria

From each of the 10^{-3} dilution, 0.1 mL of suspension had was spread onto Xylose Lysine Deoxycholate (XLD) for the isolation of *Shigella* spp. and *Salmonella* spp. and Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar plates for detecting the presence of *Vibrio spp*. For the isolation of *Pseudomonas* spp. and *Staphylococcus* spp., 0.1 mL from dilution 10^{-3} of the sample was spread on cetrimide agar and MSA agar respectively. After the incubation at 37^{o}C for 24 hrs, characteristic colonies were observed. (Cappuccino and Sherman, 1996). Finally, a series of biochemical tests were performed following the standard methods to confirm the pathogenic identification (Cappuccino and Sherman, 1996) (Table 1).

2.5 Determination of antimicrobial susceptibility

All the isolates were tested to observe their antibiotic susceptibility pattern against the 10 antibacterial drugs (including first, second and third-generation drugs) by disc diffusion assay on Mueller-Hinton Agar (Difco, Detroit, MI) with antibiotic discs (Neo-Sensitabs, Rosco, Denmark) according to the modified Kirby-Bauer method (Bauer *et al.*, 1966; Ferraro *et al.,* 2001; Munshi *et al*., 2012). A single colony of each isolate was inoculated into 2 mL of Mueller-Hinton broth and incubated at 37°C for 4 hrs. The culture turbidity was then adjusted to a 0.5 McFarland standard. Sterile cotton swabs were dipped into the suspensions and spread evenly over the entire surface of Muller-Hinton agar. Antibiotic discs of appropriate concentrations (Neomycin 10 µg, Chloramphenicol 10 µg, Polymyxin B 30 µg, ofloxacin 5 µg, amoxicillin 10µg, ciprofloxacin 5 µg, cefpodoxime 30µg, nalidixic acid 30 µg, imipenem 10 µg, tetracycline 30 µg,) were placed aseptically over

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Table 1. Biochemical tests of different pathogens

Pathogenic microorganisms	TSI				Motility	Indole Production	MR	VP	Citrate utilization	Catalase	Oxidase
	Slant	Butt	Gas	H_2S							
E. coli	Y	Y	+	-	+	+	+	-	-	+	-
Shigella spp.	R	Y	-	-	+/-	+	-	-	-	-	-
Klebsiella spp.	Y	Y	+	-	+	-	-	-	+	+	-
Vibrio spp.	R	Y	-	-	+	-	+	-	-	+	+
Staphylococcus spp.	Y	Y	-	-	+	-	+	-	-	+	-
Pseudomonas spp.	R	Y	-	-	+	-	+	-	-	+	+
Salmonella spp.	R	Y	-	+	+	-	+	-	-	+	-

All the experiments have been done three times and the results were reproducible. One representative data have been shown. TSI: Triple Sugar Iron Test, Y: Yellow (Acid), R: Red (Alkaline), MR: Methyl red, VP: Voges-Proskauer

the surface at appropriate spatial distance of 5 mm. Plates were then inverted and incubated at 37°C. After 24 hours, plates were examined and the diameters of the zones of inhibition were measured and interpreted as susceptible, intermediate and resistant.

3. Results and discussion

Fish is extremely susceptible to microbial contamination because of their soft tissues and the aquatic environment. Millions of bacteria are present in the surface of slime, on the gills and in the intestines of live fish. Many of them become potential spoilers after the death of fish when the defense system breaks down and the bacteria multiply and invade the flesh. One of the major factors contributing to poor quality of the fish in retail trade is unhygienic handling, improper storage, physical damage and come to contact in dirty water and microorganisms. Bacterial spoilage is characterized by softening of the muscle tissue and the production of slime and offensive odors. Data is represented in Figure 1.

The highest total viable bacterial (TVB) load was observed in raw *Penaeus monodon* which is 2.5×10^6 CFU/g where among the raw fishes the lowest count of TVB was recorded in *Brama brama* which is 2.7×10^5 CFU/g. However, all dried fish samples exhibit comparatively lower bacterial load than that of their corresponding raw fishes. Fungal growth was observed in all experimental raw and dried fishes. Highest fungal load was observed in raw *Penaeus monodon* whereas surprisingly fungal load was nill in case of dry *Penaeus monodon*. Total fecal coliform was absent in all samples whereas *Escherichia coli* was present in all samples which reflects the previous study of Saritha *et al.* (2012). In case of raw *Harpadon nehereus* a higher load of *E. coli* was reported (5.2×10^4 CFU/g) and lowest count of *E. coli* was recorded in *Brama brama* and that was 2.51×10^3 CFU/g. *Klebsiella* spp. was present in raw and dry *Penaeus monodon*, raw and dry *Puntius chola* and raw *Amblypharyn godonmicrolepis* and their count was recorded 6.2×10^3 CFU/g, 1.5×10^2 CFU/g, 2.51×10^2 CFU/g, 2.6×10^4 CFU/g and 2.2×10^3 CFU/g respectively. *Staphylococcus* spp. was present in Dry *Penaeus monodon*, raw and dry *Brama brama* and raw *Abmblypharyn godonmicrolepis*. *Shigella* spp. was only found in raw *Brama brama* and the count is 5.6×10^3 CFU/g. A significant load of *Salmonella* spp. was observed in almost all samples. *Salmonella* spp. was found in raw *Penaeus monodon*, raw *Harpadon nehereus*, raw *Brama brama,* raw and dry *Puntius chola* and the count was 2.5×10^2 CFU/g, 4.6×10^2 CFU/g, 2.7×10^2 CFU/g, 3.5×10^3 CFU/g, 6.5×10^2 CFU/g respectively. *Pseudomonas* spp. was present in all raw samples and two dry samples did not exhibit the presence of *Pseudomonas* spp. *Vibrio* spp. recorded in raw *Harpadon nehereus,* raw and dry *Brama brama*, raw *Puntius chola.*

For most of the pathogenic isolates, higher rates of resistance were found against imipenem (10 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), chloramphenicol (10 µg), nalidixic acid (30 µg), Neomycin (Table 2). On the other hand, most of the isolates were found to retain higher sensitivity against Tetracyclin and Amoxicillin.

In the present study, bacterial and fungal colonies were observed in the commercial sun-dried sea fishes. This may be due to post-harvest delay, improper transportation, unhygienic handling and processing during the salting and sun-drying process, contaminated working floor, salt and water. The fungal species such as *Aspergillus* sp., *Mucor* sp., *Rhizopus* sp. and *Fusarium* sp. are pathogenic to the human beings (Sharma, 1989; Felicia and Jamila, 2003) and reported to cause food spoilage. *Vibrio* spp. is a halophilic bacterium usually present in the marine environment but in the case of *Salmonella* spp., it does not occur naturally in marine

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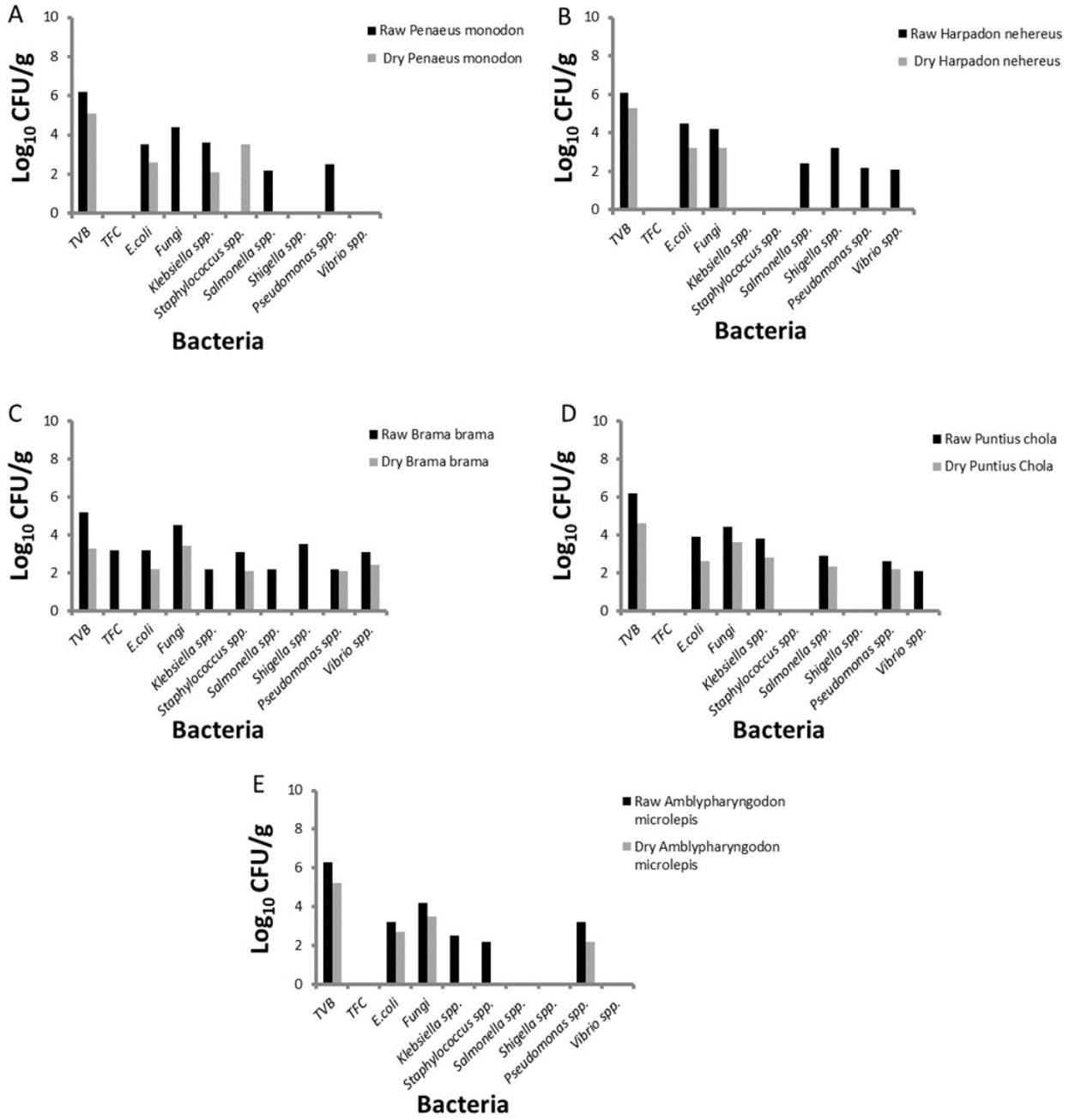

Figure 1. Comparative microbiological analysis of raw fishes and dry fishes

Table 2. Antimicrobial susceptibility pattern of different pathogenic isolates isolated from the dry and raw fish samples

Organisms	*E. coli*		*Klebsiella* spp.		*Shigella* spp.		*Salmonella* spp.		*Pseudomonas* spp.		*Staphylococcus* spp.		*Vibrio* spp.	
Antibiotics	R	S	R	S	R	S	R	S	R	S	R	S	R	S
CIP (5µg)	33%	67%	2%	98%	10%	90%	20%	100%	30%	70%	ND	ND	0%	100%
CPD(30µg)	80%	20%	100%	0%	2%	98%	20%	80%	34%	66%	ND	ND	100%	0%
AMO(10µg)	33%	67%	87%	12%	17%	83%	4%	96%	80%	20%	100%	1%	100%	0%
IPM (30µg)	10%	90%	0%	100%	9%	91%	16%	84%	20%	80%	ND	ND	0%	100%
N (10µg)	73%	27%	60%	40%	ND	ND	80%	20%	ND	ND	ND	ND	0%	100%
CHL(10µg)	45%	55%	24%	76%	52%	48%	48%	52%	66%	34%	ND	ND	100%	0%
TE (30 µg)	20%	80%	18%	82%	1%	99%	20%	80%	20%	80%	30%	70%	100%	0%
PB (30µg)	80%	20%	ND	ND	ND	ND	20%	80%	ND	ND	ND	ND	0%	100%
NA(30µg)	80%	20%	75%	25%	99%	1%	20%	80%	40%	60%	ND	ND	0%	100%
OFL (5µg)	70%	30%	ND	ND	ND	ND	ND	ND	ND	ND	22%	78%	100%	0%

S – susceptibility, R – resistance, ND – not done, (CIP – ciprofloxacin, CPD-cefpodoxime, AMO-amoxacilin, IMP-imipenum, N -Neomycin ,CHL-Chloramphenicol, PB-Polymyxin B, Na-Nalidixic acis, OFL-ofloxacin, TE-tetracycline)

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water and its presence is usually due to unhygienic handling, carriers, or polluted Halo tolerant fungi in salted and dried fish at coastal water (Chakrabarty and Varma, 1990). Dried fishes became contaminated when transferred to the retail market. Lack of proper packaging, insufficient drying and sanitary practices induces the rate of contamination (Paul *et al*., 2018). Sun -drying might reduce the microbial load in fish flesh but do not eliminate completely contaminants in most samples. In a similar, it was concluded that the poor quality of the sun-dried fishes may develop due to unhygienic processing, inadequate salting with poor quality salt and causality in case of packing of the fishes (Prakash, 2011).

4. Conclusion

Our present study revealed that both raw fish and sun -dried fish may nurture various pathogenic microorganisms. The microbial stability of dried fish products depends upon their moisture content. To control the flies, insects or pests, pesticide applied on the fish which is hazardous to the dry fish consumers, so fishermen should be aware of these things. Proper drying procedure is mandatory for achieving a high quality of dried fish. Very recently, the consumption of dry fish is not only popular to the Bangladeshi people but also getting its popularity to the people of Europe, the US and the Middle East. To improve the quality and ensure consumer acceptance it is obvious to run a training session for the dried fish processors and dried fish traders. In order to minimize the existence of drug resistant microflora in both the raw and dry fish, the fish processing company should have to be more careful about the cross contamination during the whole fish processing procedure. Government body should take necessary steps to improve the quality and safety of both raw and sun-dried fish produced in the coastal region of Bangladesh as well as to resist the frequent spreading of drug resistant microflora.

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