Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(6): 1937 – 1958 (2024)
www.ejabf.journals.ekb.eg

Microbial Quality Assessment of Traditionally Processed Fishery Products from Retail Fish Markets of Gazipur District in Bangladesh

Murshida Khan[1*], Dulan Sarker[1], Dinesh Chandra Shaha[2], Julie Lively[3]

[1]Department of Fisheries Technology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh
[2]Department of Fisheries Management, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh
[3]School of Renewable Natural Resources, Louisiana State University Agricultural Center, LA, USA
[*]**Corresponding Author: murshida@bsmrau.edu.bd**

ARTICLE INFO

Article History:
Received: Sept. 23, 2024
Accepted: Nov. 12, 2024
Online: Dec. 19, 2024

Keywords:
Citrobacter freundii, *Leclercia adecarboxylata*, *Pseudomonas aeruginosa*, *Providencia stuartii*, *Enterobacter cloacae*, *Klebsiella pneumonia*

ABSTRACT

The present study aimed to evaluate the bacterial quantity and quality of traditionally processed fishery products in Bangladesh, which are popular food items and an important source of animal protein. These products included dried bombay duck (*Herpadon nehereus*), dried ribbon fish (*Trichiuris haumela*), dried small shrimp (*Metapenaeus* sp.), salted hilsha (*Tenualosa ilisha*), and semi-fermented chepa (*Puntius sophore*) from vendors at the Chowrasta fish market in the Gazipur district. The study focused on determining the total plate count (TPC) of bacteria, moisture content, and the presence or absence of *Salmonella* and other pathogenic bacteria in these products. The TPCs ranged from 4.74 ± 3.74 to 5.08 ± 4.27 Log10 CFU/g, with the highest value found in dried bombay duck and the lowest in salted hilsha. The TPC for all five products was within the acceptable food safety limits, and *Salmonella* was not detected in any of the processed products. However, the products contained other pathogenic bacteria, including *Citrobacter freundii* and *Leclercia adecarboxylata* in the bombay duck, *Pseudomonas aeruginosa* in hilsha, *Providencia stuartii* in shrimp, *Enterobacter cloacae* in chepa, and *Klebsiella pneumoniae* in the ribbon fish. This study is the first to report the presence of *Leclercia adecarboxylata* and *Citrobacter freundii* in the dried bombay duck and *Pseudomonas aeruginosa* in salted hilsha. Although the TPCs were within acceptable limits, indicating that the products were microbiologically safe in terms of total bacterial load, while the presence of pathogenic bacteria means they could still pose a risk to human health. Therefore, while the TPC did not exceed safety thresholds, the products were not entirely safe for consumption due to the presence of harmful pathogens. The results suggest that future studies on food safety should not solely rely on TPC as a marker for safety but should also consider the presence of specific pathogenic bacteria. In conclusion, while these traditionally processed fishery products appeared to be safe based on their TPC, the presence of pathogenic bacteria highlights a need for more comprehensive food safety assessments to ensure consumer health.

INTRODUCTION

Fish is very perishable and quickly loses freshness due to autolysis, microbial attack and lipid rancidity **(Dehghani *et al.*, 2018; Rasul *et al.*, 2022a)**. In a tropical

country like Bangladesh, fish spoilage is accelerated by hot weather. Due to inadequate facilities, some fishes are processed by traditional processing method such as drying, salting, and fermentation **(Adeyeye *et al.*, 2015)**. Processing techniques are applied to maintain quality and increase shelf life **(Rasul *et al.*, 2022b)**. Due to the storage facility limitations during peak harvest season, traditionally processed fishery products not requiring cold storage are produced. Dried fish are processed from October to March, salted products (e.g. salted hilsha) are processed during the monsoon season, and semi-fermented fishery products (e.g. Chepa shutki) are processed during the winter season **(Nowsad, 2007)**. Although Bangladeshi people enjoy different traditionally processed fish products, quality and food safety of those traditionally processed fish product is a significant consideration.

Some bacteria are natural microflora in fish and aquatic environments, and some bacteria are present in fish due to cross-contamination during handling. In the United States, Australia, New Zealand and Hong Kong, the legal limit of *Salmonella*, *Listeria monocytogenes*, and *Vibrio cholerae* is zero in 25g of raw or cooked fish **(Norhana *et al.*, 2010)**. In the EU, the second major cause of food-borne disease is *Salmonella* **(European Food Safety Authority, 2011)**. *Salmonella* is responsible for salmonellosis disease **(Ray & Bhunia, 2007)**. In 2022, shrimp from Bangladesh imported to the US were rejected due to *Salmonella* spp. **(FDA, 2022)**.

For exported shrimp, processors of Bangladesh are very conscious about the presence of *Salmonella*, but the status of *Salmonella* in traditionally processed fish products for domestic consumption is often ignored. A few studies have looked at microbial quantity and nutritional quality of traditional fishery products in Bangladesh with mixed results when it comes to food safety. Other pathogenic bacteria can be present in the processed fishery products in retail market such as *Staphylococcus aureus, S. epidermidis, Bacillus cereus, B. subtilis, B. megaterium, Micrococcus luteu, Klebsiella* sp., and *Pseudomonas* sp. **(Sultana *et al.*, 2010; Nur *et al.*, 2020)**.

While nutrient composition and bacterial quantity of some traditionally processed fish products were studied (**Nayeem *et al.*, 2010; Sultana *et al.*, 2010; Majumdar *et al.*, 2017; Nahar *et al.*, 2017)**, *Salmonella* and pathogenic bacteria presence in traditionally processed fish products has rarely been studied in Bangladesh **(Majumdar & Rashid, 2017; Rasul *et al.*, 2020)**. Additionally, most previous studies collected samples from the processors and not from the retail markets, which could also be a source of bacterial contamination. When it comes to consumer safety, the final product a consumer purchases needs to be safe. Traditionally processed fishery products can be a major carrier of *Salmonella* and other pathogenic bacteria as they can be introduced by contaminated water and unhygienic handling **(Sant'Ana, 2012)**. Therefore, the goal of

this research was to investigate the microbial quantity, moisture content and status of other pathogenic bacteria in some traditionally processed fishery products (dried bombay duck, dried ribbon fish, salted hilsha, small shrimp and chepa shutki) from fish markets in Gazipur, Bangladesh.

MATERIALS AND METHODS

1. Sources of traditionally fishery products

Traditionally processed fishery products including 3000g dried bombay duck (*Herpadon nehereus)*, 3000 g dried ribbon fish (*Trichuris haumela)*, 500g dried shrimp (*Metapenaeus* spp.), 500g semi fermented chepa (*Puntius* spp.), and 3000g salted hilsha (*Tenualosa ilisha*) were collected from two fish markets of the Gazipur district between December 2018 and May 2019. All samples were collected in sterile plastic bags and stored at 4°C. For all products except salted hilsha, 10 samples (each sample contained 100g) were taken at random. For salted hilsha, 10 samples (each sample was 250-450g wt.) were taken. All samples were run in triplicate. Experiments were performed at the Department of Fisheries Technology Lab, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh.

2. Determination of moisture

The moisture content was determined by **AOAC (1990)** method. In the study, approximately 5g of each fishery product sample was ground homogeneously and placed in a weighed aluminum plate. The plate was then weighed using an electric balance (AL54 analytical balance, Mettler Toledo). The sample was dried in a hot air oven (US: Isotemp oven, Fisher Scientific; BD: Precision™, Thermo Fisher Scientific) at 105°C for 24 hours, ensuring that the weight remained consistent. After drying, the plates were placed in a desiccator to cool before taking the final weight of the shrimp.The moisture content of each sample was then calculated using the formula: Moisture (%) = ((Weight of sample – Weight of dried sample) / Weight of sample) × 100. This process was repeated for three replicates for each sample, and the average moisture content was calculated.

3. Determination of total plate count of bacteria

The aerobic mesophilic bacterial load was estimated using the method described in Bacteriological Analytical Manual (BAM) **(Maturin & Peeler, 2001)**. Countable plates showing 25 to 250 colonies were selected and counted. Plates with bacterial colonies less than 25 were not standard and more than 250 value were regarded as TNTC (to numerical to count).

4. Determination of *Salmonella*

Salmonella testing followed the standard bacteriological analytical manual **(Andrews *et al*., 2000)**. Aseptically, a 25g sample was placed in a sterile bottle, and 225ml of lactose broth was added and mixed by shaking for 60s. The sample was homogenized using a sterile mortar and pestle. The homogenized mixture was aseptically transferred to sterile, wide-mouth laboratory bottle (500ml) and kept for 60min at room temperature. The sample solutions were thoroughly mixed by swirling, and the pH of the solutions was measured using a pH meter. The pH was then adjusted to 6.8 ± 0.2. After pH adjustment, the bottles containing the samples were placed in an incubator and incubated for 24 ± 2 hours at 35°C. Following the incubation period, 0.1mL of the mixture from each sample was added to 10mL of Rappaport-Vassiliadis (RV) medium and vortexed. The RV medium was then incubated in a water bath at 42 ± 0.2°C for another 24 ± 2 hours. After incubation, the RV medium was vortexed again, and a 3mm loopful of RV broth was streaked onto xylose lysine desoxycholate (XLD) agar (Sigma). The XLD petri dishes were then incubated for 24 hours at 35°C. After the incubation, the petri dishes were examined for the presence of *Salmonella* colonies, indicating whether *Salmonella* was present in the samples.

5. Determination of other pathogenic bacteria

Serially diluted 100µL of the diluted (10^{-3} to 10^{-7}) samples were spread on plate count agar (Himedia, India) and incubated at 37°C for 24h. The isolates were systematically sub-cultured on nutrient agar, and stock cultures were maintained in NB (Nutrient Broth) supplemented with 10% glycerol and were stored in a freezer at -80°C before molecular identification.

6. Molecular identification of bacteria

Molecular identification followed five steps: isolation of genomic DNA, DNA quality measurement by gel electrophoresis, amplification by PCR, purification of PCR sample and finally DNA sequencing of isolated bacteria **(Hannan *et al*., 2019)**. Susceptive bacterial colonies were taken from pure culture stock, inoculated into a nutrient broth (Liofilchem), and incubated in a shaker incubator (120rpm) at 28°C for 24-48 hours. After incubation, bacterial colonies were used for genomic DNA extraction. GeneJET Genomic DNA purification kit # K0721 (Thermo scientific) protocol was used for genomic DNA extraction. Electrophoresis was used to check the DNA quality by comparing it with the 1 Kb plus DNA ladder marker (Thermo Fisher Scientific, USA), and the DNA band was observed under the UV light (UVDI, Major Science).

The polymerase chain reaction (PCR) with universal primer sets was used for amplification (forward primer 8F, 5’-AGAGTTTGATCCTGGCTCAG-3’ and reverse primer 1492R, 5’-AGGAGGTGATCCAACCGCA-3’ (Thermo Fisher Scientific, USA)).

The PCR thermocycler (2720 thermal cycler, Applied Biosystems) was used for amplification. The thermal profile for PCR included an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 57°C for 40 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes, and the PCR products were visualized using agarose gel electrophoresis and a gel documentation system under UV light.

The PCR product was purified using a commercial PCR purification kit (Thermo Scientific GeneJET PCR Purification Kit #K0701), and the purified PCR product was stored at -20 °C for further use. The purified PCR products with sequencing primer were sent to the National Institute of Biotechnology, Savar, Dhaka, to sequence the 16S rRNA gene. After getting sequencing results, they were searched using BLAST (Basic Local Alignment Search Tool) at the National Center for Biotechnology Information website (NCBI, http://www.ncbi.nlm.nih.gov/).

7. Data analysis

A Kruskal-Wallis test was used to test for significant differences for each moisture content and total plate count among different species (R version 4.111). A Tukey's HSD test was performed for indicating significance at $P<0.05$. All results were reported as mean ± SD of triplicate measurements.

RESULTS AND DISCUSSION

1. Moisture content

1.1. Dried fish (bombay duck, ribbon fish and shrimp)

The dried bombay duck had a moisture content of 27.07 ± 0.77% (ranging from 26.07% to 27.99%), dried ribbon fish had a moisture content of 34.13 ± 0.87% (ranging from 32.82% to 35.19%), and dried shrimp had a moisture content of 20.35 ± 0.74% (ranging from 19.08% to 21.41%) (Table 1) (Fig. 1). The moisture content of small shrimp was closest to the recommended food safety range of less than 15-16% **(Nowsad, 2007)**, while the moisture content in bombay duck and ribbon fish exceeded this recommended rate. Previous studies found that sun-dried fish typically contains 10 to 20% moisture **(Haque, 2004)**, with dried bombay duck containing 15.25% moisture **(Haque *et al.*, 2013)**. **Azam *et al.* (2003)** found a moisture content of 21.26% in dried bombay duck, which is still lower than the values observed in the present study. Additionally, other research indicated that the moisture content of dried ribbon fish ranged from 14.06% to 24.90% **(Azam *et al.*, 2003; Flowra *et al.*, 2012; Imtiaz *et al.*, 2017)**, much lower than the 34.13% moisture content found in our ribbon fish samples. The relatively higher moisture content in our dried shrimp (20.35%) is likely due to the higher demand for fresh shrimp in Bangladesh, leading to less attention on dried shrimp

products. Most of the microbiological and enzymatic activities are slowed down or stopped when the moisture content is less than 15-16% in the final product **(Nowsad, 2007)**. The high moisture content indicates that there is a high risk of the growth of microorganisms due to improper drying or storage **(Akter *et al*., 2018)**.

Table 1. Moisture content and total plate count (TPC)

Sample Name	Moisture (%)	TPC (Log10Cfu/g)
Dried bombay duck (*Herpodon neherus*)	27.07 ± 0.77	5.08 ± 4.27
Dried ribbonfish (*Trichiuris haumela*)	34.13 ± 0.87	4.90 ± 0.07
Dried shrimp (*Metapenaeus spp.*)	20.36 ± 0.74	4.95 ± 3.81
Salted hilsha (*Tenualosa ilisha*)	21.07 ± 0.73	4.74 ± 3.74
Semi-fermented chepa (*Puntius sophore)*	41.26 ± 0.82	5.07 ± 4.20

3.1.2. Salted hilsha

Moisture content of salted hilsha was 21.07 ± 0.73% (20.06 to 21.96%) (Table 1) (Fig.1). Previous research reported moisture content in salted hilsha at 23.9% to 54.35% **(Azam *et al*., 2003; Majumdar *et al*., 2004; Majumdar & Basu, 2010; Kaiser *et al*., 2017; Tabassum *et al.*, 2018)**. This broad range could be due to storage condition, seasonal variation, processing method, or storage time which can all influence moisture content. Another study found a significant decrease in moisture content in salted hilsha during six days of storage from 63.79 to 47.65% **(Mukti *et al*., 2016)**.

3.1.3. Semi-fermented chepa

The moisture content of semi–fermented chepa was 41.26 ± 0.82% (40.11 to 42.64%) (Table 1 & Fig.1). **Nahar *et al.* (2017)** observed a moisture content range of 33.74 to 45.03% in semi-fermented chepa from a retailer, while another study found the moisture content ranged from 39.62 to 46.89% **(Nayeem *et al*., 2010)**. When semi-fermented chepa was stored at 18 to 34°C for 120 days, the moisture content was 34.02% **(Mahanta & Muzaddadi, 2012)**. In this experiment, the moisture content was higher,

and the reasons may be storage time, absorbing moisture from environment, and the intentional or unintentional addition of water to increase product weight **(Nayeem *et al.*, 2010)**. In retail it is difficult to know the actual storage time. In the present experiment, the moisture content is not preferable as this is suitable for the growth of microorganisms.

There was a significant difference of moisture content among different species (Fig. 1; *P*=0.000000012). All four products were significantly different except Dried small shrimp with salted hilsha (Fig. 1). Among five of the fishery products, dried ribbon fish and semi-fermented chepa had the highest moisture content while dried shrimp and salted hilsha had the lowest, although still high from a microbial growth standpoint. Maintaining proper techniques during drying, storage and retail is important for maintaining low moisture, and therefore, increasing food safety.

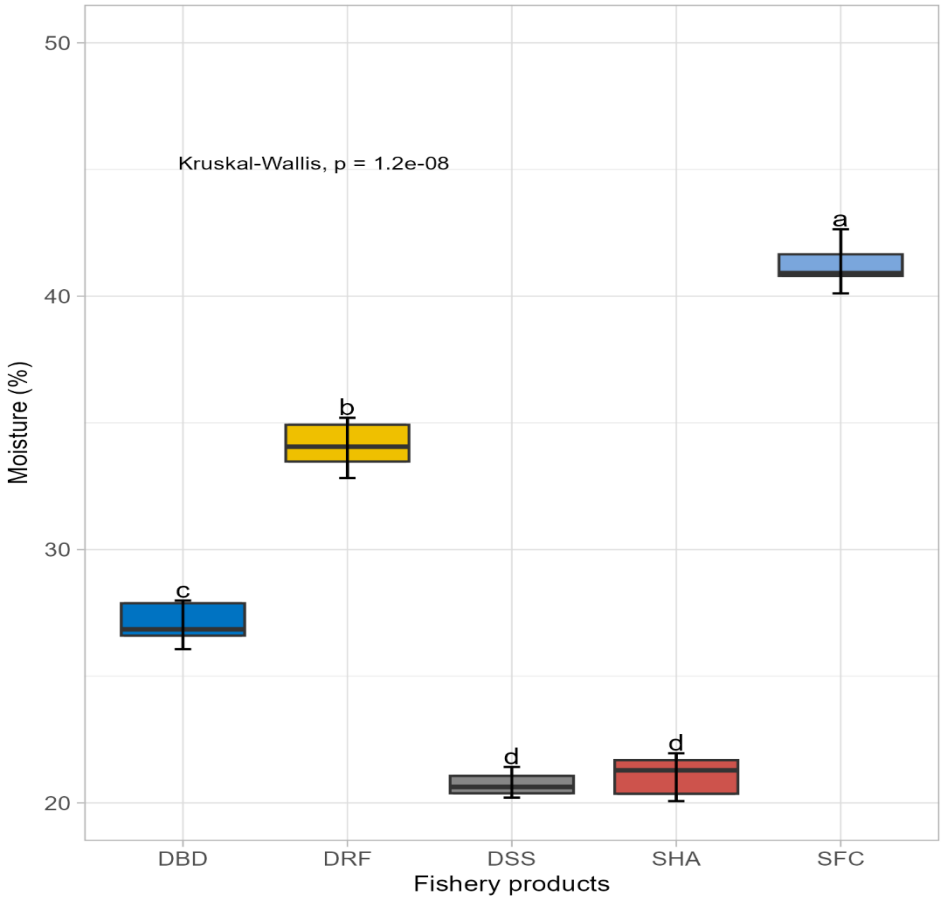

Fig. 1. Moisture content of different fishery products. Different letters indicate significant difference; all products were different from each other (ANOVA; *P*= 0.000000012). DBD= Dried bombay duck, DRF= Dried ribbonfish, DS= Dried shrimp, SHA=Salted hilsha, SFC= Semi-fermented chepa

3.2 Total plate count (TPC) of bacteria

3.2.1 Dried fish (bombay duck, Ribbon fish and small shrimp)

The TPC value of bombay duck was 5.08 ± 0.061 Log10 CFU/g (5.01 to 5.22 Log10 CFU/g) (Table 1 & Fig. 2). Previous work found the TPC of the dried bombay duck collected from Chakti and Cox's bazar region were 3.82 Log10 CFU/g and 3.59 Log10 CFU/g **(Haque *et al*., 2013)**. The dried bombay duck from fish markets of Chittagong and Mymensingh had TPC values of 2.53 Log10 CFU/g to 2.58 Log10 CFU/g **(Sultana *et al*., 2010)**. In this experiment, the TPC value was higher than known previous results, which could be due to moisture content being higher than recommended values. Other possible reasons for the higher moisture content include regional variations, spoilage of fish before drying, drying the fish on the ground or in other unhygienic locations, or inadequate washing of the fish before the drying process.

The TPC value of the dried ribbon fish was 4.89 ± 0.07 Log10 CFU/g (4.78 to 5 Log10 CFU/g) (Table 1 & Fig. 2). **Imtiaz *et al*. (2017)** found that the TPC value of the dried churi fish was 5.47 Log10 CFU/g after seven days of storage and observed that the bacterial load was decreasing gradually. However, in the markets of the Chittagong and Mymensingh regions, the dried ribbon fish had a total plate count of 3.95 Log10 CFU/g from Anwara fish market, 3.91 Log10 CFU/g from Baskhali market and 3.70 Log10 CFU/g from Karnafuli Khamar market. In this experiment, TPC value was higher compared to previous results, and it may be due to region variation, improper drying and moisture absorption from the surrounding environment during storage.

The TPC of dried small shrimp in our study was 4.95 ± 0.03 Log10 CFU/g (4.90 to 5.00 Log10 CFU/g) (Table 1 & Fig. 2). No other previous work is known to compare values. According to **ICMSF (1986)**, a TPC of less than 6-7 Log10 CFU/g is considered suitable for human consumption. Variations in TPC from previous studies are natural, as microorganism numbers depend on many factors. In terms of safety, the TPC of dried bombay duck, ribbon fish, and small shrimp were within acceptable limits and deemed safe for human consumption.

3.2.2. Salted hilsha

The TPC value of salted hilsha was 4.74 ± 0.04 Log10 CFU/g (4.69 to 4.84 Log10 CFU/g) (Table 1 & Fig. 2). **Kaiser *et al.* (2017)** studied the bacterial load of three types of salted hilsha samples collected from retail market of Mymensingh, and the TPC was 6.26 Log10 CFU/g, with a moisture content of 40.03 to 43.09%. Another study looked at the TPC for fresh (Day 0) and salted hilsha on Day 6 and found it changed from 5.96 Log10 CFU/g for fresh hilsha to 8.13 Log10 CFU/g on Day 6 **(Mukti *et al*., 2016)**. The variation between their results and this study could be due to the salt content as they used 25% NaCl, but the salt quality was unknown in this experiment. **Majumdar and Rashid**

(2017) also assessed that total plate count (TPC) of dry salted hilsha collected from the retail markets of Narsingdi, Chandpur and Chittagong were 6.75 Log10 CFU/g to7.94 Log10 CFU/g and 5.34 Log10 CFU/g to 5.49 Log10 CFU/g). Normally, 25% of NaCl is suitable for good quality and maturing of the salted hilsha **(Mukti *et al*., 2016)**, but the TPC of salted hilsha also varies if it was processed with poor quality crude solar salt under poor hygienic conditions **(Kaiser *et al.*, 2017)**. In this study, the TPC for salted hilsha is considered suitable for human consumption **(ICMSF, 1986)**.

3.2.3. Semi-fermented chepa

The TPC value of semi-fermented chepa was 5.07 ± 0.059 Log10 CFU/g (5 to 5.16 Log10 CFU/g). **Nahar *et al*. (2017)** noticed that the total bacterial count of semi-fermented chepa ranged from 6.01 to 8.10 Log10 CFU/g. On the other hand, **Mahanta and Muzaddadi (2012)** found that the total bacterial count in semi-fermented chepa fish was 7 Log10 CFU/g within 120 days of storage. Our results were lower than these known studies, but the value was acceptable for human consumption **(ICMSF, 1986)**.

The ANOVA results indicated significant different in TPC between the five products (P=0.000000015) (Fig. 2). The dried bombay duck was similar to semi-fermented chepa (P= 0.77) with the highest TPC. Dried ribbon fish and dried small shrimp (P = 0.064) were significantly lower than the bombay duck and chepa, but also not significantly different from each other. Salted hilsha has significantly the lowest TPC (Fig. 2). TPC can vary due to raw material condition, processing methods, surrounding environment condition, and cross contamination **(Nowsad, 2007)**. Traditional drying of fishes is often done in the open field or on the sand, and there are chances of contamination with sands and other particles **(Flowra *et al*., 2012)**. The dried products are often of lower quality due to varying temperature levels and contamination of the products with dust, vermin's and leafs **(Reza *et al*., 2005)**. The quality of the salt and surrounding environment could also affect the salted hilsha TPC **(Kaiser *et al*., 2017)**. This experimental result indicated that marketed fishery products of Gazipur, Bangladesh are safe for consumption based on TPC.

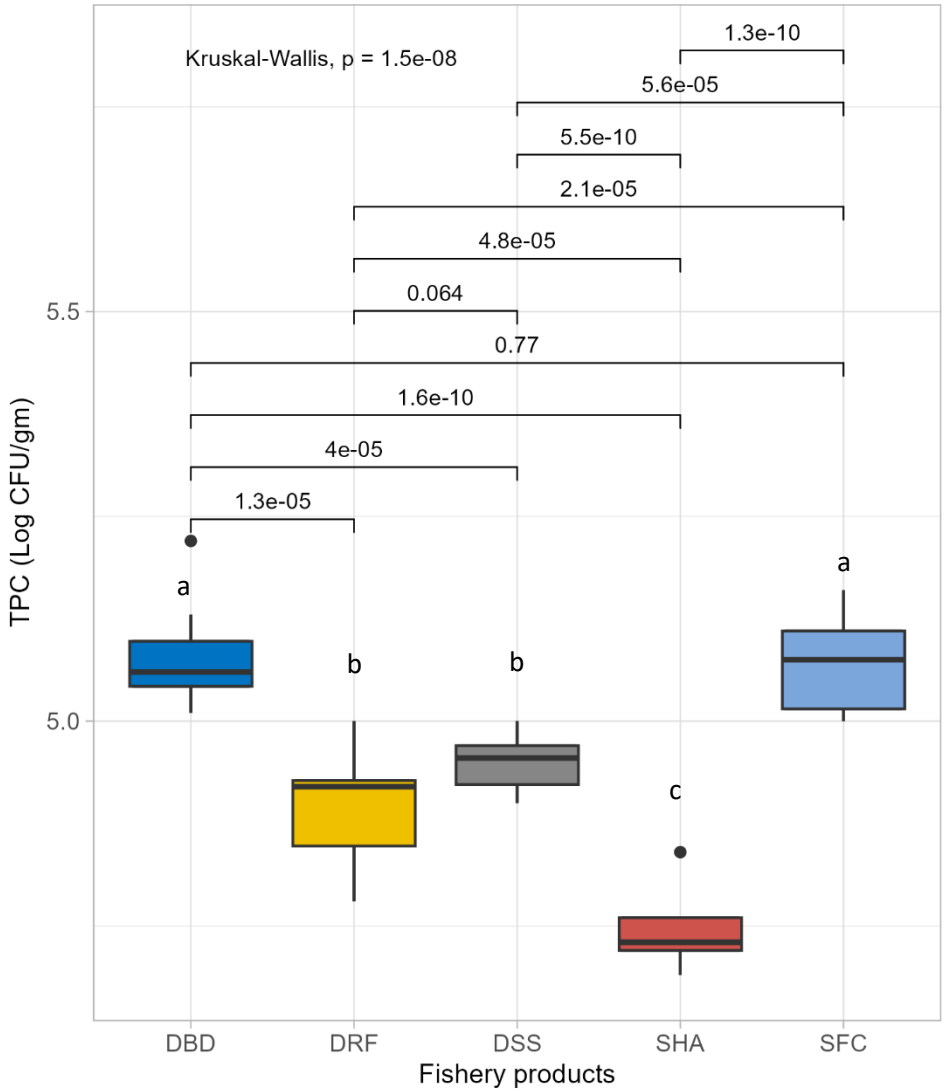

Fig. 2. Total plate count of bacteria in different fishery product. Different letters indicate significant difference (ANOVA; *P*=0.000000015). DBD= Dried bombay duck, DRF= Dried ribbonfish, DS= Dried shrimp, SHA=Salted hilsha, SFC= Semi-fermented chepa

3.3 Specific bacteria

No *Salmonella* was found. Six bacterial strains were identified from five processed fishery product during *Salmonella* isolation: *Citrobacter freundii* (bombay duck; MN625856), *Leclercia adecarboxylata* (bombay duck; MN625857), *Pseudomonas aeruginosa* (hilsha; MN625859), *Providencia stuartii* (shrimp, MN625858),

Enterobacter cloacae (chepa, MN625860), and *Klebsiella pneumonia* (ribbon fish; MN625862) (Table 2). Thirty-one total isolates were found. Isolates were found in two to seven out of the ten samples for each product (Table 2).

This is the first report of *Leclercia adecarboxylata* and *Citrobacter freundii* in the dried bombay duck fish. *L. adecarboxylata* represented 22.58% of the isolates (Table 2). Limited research has been conducted on the proximate composition or total plate count (TPC) of the dried bombay duck **(Azam *et al*., 2003; Sultana *et al*., 2010; Haque *et al*., 2013)**. *L. adecarboxylata* is an opportunistic human pathogen that phenotypically resembles Escherichia **(Stock *et al*., 2004)**. It grows at temperatures ranging from 25°C to 36°C **(Tamura *et al*., 1986)** and is widely distributed in nature, forming part of the normal flora in the gut of animals **(Stock *et al*., 2004; Tam & Nayak, 2012; Keren *et al*., 2014)**. The presence of this bacterium may indicate unhygienic conditions. *L. adecarboxylata* can cause infections in immunocompromised humans **(Hess *et al*., 2008)**. It has been found in traditional dried anchovies (*Encrasicholina punctifer*) in Oman **(Al Bulushi *et al*., 2013)**, and multidrug-resistant *L. adecarboxylata* has been detected in the rivers and lakes of Dhaka City **(Haque *et al*., 2015)**. Recently, this species was also found in tilapia (*Oreochromis niloticus*) collected from retail fish markets **(Khan *et al*., 2022)**. The possible sources of *L. adecarboxylata* may include contamination from raw materials or during handling at the market.

Table 2. Bacteria isolated from fishery products. (Note: + indicates presence and – indicates absence; DBD= Dried bombay duck, DRF= Dried ribbonfish, DS= Dried shrimp, SHA=Salted hilsha, SFC= Semi-fermented chepa.)

Sl. No.	Bacterium	Accession number	Processed fishery products					Total Isolates (%)	Samples positive for isolates (out of 10)
			DBD	DRF	DS	SHA	SFC		
1.	*Leclercia adecarboxylata*	MN625857	+	-	-	-	-	7 (22.58%)	4
2.	*Citrobacter freundii*	MN625856	+	-	-	-	-	4 (12.90%)	2
3.	*Klebsiella pneumonia*	MN625862	-	+	-	-	-	3 (9.67%)	2
4.	*Providencia stuartii*	MN625858	-	-	+	-	-	3 (9.67%)	2
5.	*Pseudomonas aeruginosa*	MN625859	-	-	-	+	-	6 (19.35%)	4
6.	*Enterobacter cloacae*	MN625860	-	-	-	-	+	8 (25.80%)	7
								31 (100%)	21

C. freundii in the Bombay duck made up 12.90% of isolates (Table 2). An aerobic Gram-negative bacteria, *C. freundii* is found in the environment, food, and the intestinal tracts of animals and humans. It grows in mesophilic environments **(Wang *et al*., 2000)**. *C. freundii* causes a variety of infections, including those in the urinary tract, respiratory tract, wounds, bones, meninges, intestines and blood stream of humans. Sporadic infections and outbreaks caused by *C. freundii* were reported in 1979 in India, where seventeen babies were infected and one died **(Murray *et al.*, 2010)**. The presence of this microorganism in any fishery products is a cause for concern. It is also responsible for diseases in fish. Mass mortality of doctor fish (*Garra rufa*) was caused by *C. freundii* infection **(Baeck *et al*., 2009)**, and in infections of the Brazilian catfish (*Pseudoplatystoma*) **(Padua *et al*., 2004)**. As these bacteria are found in water, high moisture content could contribute to their spread. Maintaining hygenic conditions from every step of processing to marketing is important to minimize this bacteria.

In this research, *Klebsiella pneumonia* (9.67%) was isolated from dried ribbon fish (Table 2). *K. pneumonia* is non-motile, rod-shaped, gram-negative, opportunistic pathogen from the family Enterobacteriaceae **(Kumar *et al*., 2011)** and grows at 25°C to 42°C **(Tsuji *et al*., 1982)**. As Bangladesh is a tropical country, temperature is always around 20°C in summer and winter, which makes it a favorable environment for its growth. *K. pneumonia* has been isolated from a variety of fish and seafood including tuna sashimi implicated in an outbreak of scombroid fish poisoning in California **(Taylor *et al*., 1979)**, fresh and smoked fish *Clarias gariepinus* obtained from two markets in Benin City **(Abolagba & Igbinevbo, 2010)**, Nishikigoi *Cyprinus carpio* (carp) **(Oliviera *et al*., 2014)**, and clownfish of Maldives associated with skin hemorrhages and ulcer **(Gopi *et al*., 2016)**. The presence of this microorganism in fish and fishery products is not surprising. However, *K. pneumonia* is an opportunistic agent and caused severe cases of pneumonia in immunocompromised individuals **(Abbas *et al*., 2023)**. The presence of this organism in fishery products is not safe to eat and the presence of *K. pneumonia* could be due to the inadequate hygiene during handling **(Oliveira *et al*., 2014)**. Other possible sources of *K. pneumonia* may be cross contamination during handling, drying and improper sanitary condition. Proper hygiene practices throughout the supply chain may minimize this bacteria.

Providencia stuartii (9.67%) was isolated from dried small shrimp (Table 2). Providencia stuartii is the most common species of Providencia, a Gram-negative, facultative anaerobic bacterium belonging to the family Enterobacteriaceae. It grows best in mesophilic temperature ranges **(Sohngen *et al*., 2016)**. As Bangladesh is a tropical country, the temperature is conducive to its growth. P. stuartii is also an opportunistic pathogen, commonly found in patients with severe burns and bloodstream infections **(Lin *et al.*, 2008)**. The presence of this organism in fish is a concern for food safety. *P. stuartii*

has been found in fresh tilapia (*Oreochromis niloticus*) in Sokoto, Nigeria **(Shinkafi & Ukwaja, 2010)**. The possible sources of *P. stuartii* are similar to those of other harmful bacteria, including contamination from fresh fish at the market, unhygienic conditions, and cross-contamination during processing or from the fish market.

In this study, *Pseudomonas aeruginosa* was isolated from salted hilsha (19.35%) (Table 2). This is the first report of *P. aeruginosa* in salted hilsha in Bangladesh. *P. aeruginosa* is a Gram-negative, non-fermentative rod, aerobic, oxidase-positive, and motile with polar flagella, found in a wide range of environments. It has been isolated from numerous sources, including drinking water, domestic and wild animals, humans, plants, and various foods **(Lee *et al.,* 1999)**. *P. aeruginosa* is salt-tolerant **(Mahadevan *et al.,* 2010)**, making its presence in salted fish unsurprising. *P. aeruginosa* has been found in salmon, pangasius, shark, catfish, shrimp, and oysters in Switzerland **(Boss *et al.,* 2016)**, traditional dried anchovies (*Encrasicholina punctifer*) in Oman **(Al Bulushi *et al.,* 2013)**, both fresh and smoked *Clarias gariepinus* from two markets in Benin City **(Abolagba & Igbinevbo, 2010)**, and in fresh fish, smoked fish, and bovine meat **(Benie *et al.,* 2017)**. *P. aeruginosa* grows well at 37°C and can survive at a wide range of temperatures from 4 to 42°C **(LaBauve & Wargo, 2012)**. It can cause serious disease in immunocompromised individuals and is a common cause of nosocomial infections **(Sadikot *et al.,* 2005)**. As Bangladesh is a tropical country, the temperature is suitable for its growth, and the salt tolerance of this bacterium is also advantageous, as salted hilsha contains around 25% salt **(Mukti *et al.,* 2016)**. The presence of this organism in any fishery product is a concern, and possible sources of contamination include poor-quality salt, improper handling, improper storage, and cross-contamination.

Enterobacter cloacae from semi-fermented chepa fish represented 25.80% of the isolates (Table 2). *E. cloacae* is a Gram-negative, anaerobic bacterium belonging to the family Enterobacteriaceae, and it is widely distributed in the environment **(Wang *et al.,* 1989; Paterson *et al.,* 2005)**. It is also an opportunistic pathogen of humans **(Flyan *et al.,* 1987; Gaston, 1988)** and other organisms including fish **(Troast, 1975; Hansen *et al.,* 1990)**. *E. cloacae* has been found in the feces of humans and animals, water, soil, plants, plant materials, insects, and dairy products **(Davidson *et al.,* 2000; Neto *et al.,* 2003)**. It is capable of causing opportunistic infections **(Storti *et al.,* 2005; Mokracka *et al.,* 2011)**. *E. cloacae* was found in dead *Pangasianodon hypophthalmus* reared in a culture pond in Bhimavaram, Andhra Pradesh, India **(Kumar *et al.,* 2013)**, dead *Mugil cephalus* in India **(Thillai *et al.,* 2008)**, and traditional dried anchovies (*Encrasicholina punctifer*) in Oman **(Al Bulushi *et al.,* 2013)**.

This species thrives in mesophilic environments, with an optimal temperature of 37°C **(Thillai *et al.,* 2008)**, which favors the growth of *E. cloacae* in semi-fermented chepa fish, as the summer temperatures in Bangladesh range from 30-40°C. The use of

dirty earthen pots, improper and unhygienic handling, anaerobic conditions, moisture absorption from the surroundings, and unsanitary market conditions are additional potential contributing factors. The discovery of *E. cloacae* in semi-fermented chepa fish has never been reported before.

Moisture content plays a significant role in bacterial growth. Typically, processed fishery products contain 16-18% moisture. In this experiment, all the processed fishery products contained more than 16% moisture, creating conditions that support bacterial growth. Possible reasons for high moisture content include absorption from the surrounding environment, inadequate drying during processing, or improper storage between marketing and distribution.

Citrobacter freundii, *Leclercia adecarboxylata*, *Pseudomonas aeruginosa*, *Providencia stuartii*, *Enterobacter cloacae*, and *Klebsiella pneumoniae* were found in various samples at temperatures conducive to a tropical climate like Bangladesh. Most of these bacteria have previously been identified in fresh fish. Traditionally processed products include dried, salted, and semi-fermented fish. This is the first report of these pathogenic bacteria being present in processed fishery products in Bangladesh. All of these bacteria are human pathogens. Possible causes of contamination include improper handling, cross-contamination during processing, inadequate drying, improper storage, the use of low-quality solar salt, contamination during fermentation, and cross-contamination at the markets. By maintaining hygienic conditions throughout the supply chain and ensuring proper cold storage, the presence of these bacteria may be reduced or eliminated.

CONCLUSION

Studies were conducted to investigate the moisture content, total plate count (TPC), and bacteriological status (including *Salmonella* and other pathogenic bacteria) of five processed fishery products from the retail market in Gazipur district. Higher moisture content was found in all products, exceeding the recommended value for food safety. TPC levels were within the acceptable food safety ranges for all products. Six bacterial isolates were identified: *Citrobacter freundii*, *Leclercia adecarboxylata*, *Pseudomonas aeruginosa*, *Providencia stuartii*, *Enterobacter cloacae*, and *Klebsiella pneumoniae*. Based on TPC results, these products are deemed safe for human consumption; however, the six isolated bacterial strains are harmful to humans. Future food safety research should focus not only on moisture content and TPC but also on improving the quality of processed fishery products in Bangladesh to ensure consumer safety.

REFERENCES

Abolagba, O. J. and Igbinevbo, E. E. (2010). Microbial load of fresh and smoked fish marketed in Benin metropolis, Nigeria. Res. J. Fish. Hydrobiol., 5(2): 99-104.

Adeyeye, S. A. O.; Oyewole, O. B.; Obadina, A. O. and Omemu, A. M. (2015). Microbiological assessment of smoked silver catfish (*Chrysichthys nigrodigitatus*). Afr. J. Microbiol. Res., 5: 1–9.

Akter, T.; Sayeed, M. A.; Rasul, M. G.; Kashem, M. A. and Paul, A. K. (2018). Evaluation of microbiological quality of dried baim (*Mastacembelus armatus*) in Bangladesh. AAES, 3(4): 344-353.

Al Bulushi, I. M.; Guizani, N. and Dykes, G. A. (2013). Effect of ambient storage on the microbial characteristics of traditional dried anchovies (*Encrasicholina punctifer*). Afr. J. Microbiol. Res., 7(28): 3575-3581.

Andrews, W. H.; Wang, H., Jacobson, A. and Hammack, T. (2000). Laboratory methods - bacteriological analytical manual (BAM) Chapter 5: *Salmonella*. Retrieved August 20, 2018, from https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm

AOAC (1990). Official methods of analysis. *Association of Official Analytical Chemists, Arlington, VA.*

Azam, K.; Basher, M. Z.; Asaduzzaman, M.; Hossain, M. H. and Ali, M. Y. (2003). Biochemical quality assessment of fourteen selected dried fish. Univ. J. Zool. Rajshahi Univ, 22: 23-26.

Baeck, G. W.; Kim, J. H.; ChorescaJr, C.; Gomez, D. K.; Shin, S. P.; Han, J. E. and Park, S. C. (2009). Mass mortality of doctor fish (*Garra rufa obtusa*) caused by *Citrobacter freundii* infection. J Vet Clin., 26(2): 150-154.

Benie, C.K.D.; Dadié, A.; Guessennd, N.; N'gbesso-Kouadio, N.A.; Kouame, N.Z.D.; N'golo, D.C.; Aka, S.; Dako, E.; Dje, K.M. and Dosso, M. (2017). Characterization of virulence potential of *Pseudomonas aeruginosa* isolated from bovine meat, fresh fish, and smoked fish. Eur. J. Microbiol. Immunol., 7(1): 55-64.

Boss, R.; Overesch, G. and Baumgartner, A. (2016). Antimicrobial resistance of *Escherichia coli*, Enterococci, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* from raw fish and seafood imported into Switzerland. J. Food Prot., 79(7): 1240-1246.

Carpenter, J. L. (1990). *Klebsiella* pulmonary infections: occurrence at one medical center and review. Rev. Infect. Dis., 12(4): 672-682.

Davidson, E. W.; Rosell, R. C. and Hendrix, D. L. (2000). Culturable bacteria associated with the whitefly, *Bemisia argentifolii* (Homoptera: Aleyrodidae). Fla. entomol., 159-159.

Dehghani, S.; Hosseini, S. V. and Regenstein, J. L. (2018). Edible films and coatings in seafood preservation: A review. Food Chem., 240: 505–513. https://doi.org/10.1016/j.foodchem.2017.07.034

European Food Safety Authority (2011). the European Union summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in 2009, efsa j., 9(3):2090,1-378.

Fetriyuna, F.; Nurhasanah, S.; Ristiani, E. and Devitasari, C. (2017). Characteristics and shelf life of dry salted and unsalted dark banded goatfish (*Upeneus* sp.). KnE life sci., 471-481.

Flowra, F. A.; Tumpa, A. S. and Islam, M. T. (2012). Biochemical analysis of five dried fish species of Bangladesh. Univ. J. Zool. Rajshahi Univ., 31: 09-11.

Flynn, D. M.; Weinstein, R. A.; Nathan, C.; Gaston, M. A. and Kabins, S. A. (1987). Patients' endogenous flora as the source of "nosocomial" Enterobacter in cardiac surgery. J. Infect. Dis., 156(2): 363-368.

Forrester, J. D.; Adams, J. and Sawyer, R. G. (2012). *Leclercia adecarboxylata* bacteremia in a trauma patient: case report and review of the literature. Surg. Infect., 13(1): 63-66.

Frank, J. A.; Reich, C. I.; Sharma, S.; Weisbaum, J. S.; Wilson, B. A. and Olsen, G. J. (2008). Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. Appl. Environ. Microbiol., 74(8): 2461-2470.

Gaston, M. A. (1988). Enterobacter: an emerging nosocomial pathogen. J. Hosp. Infect., 11(3): 197-208.

Gopi, M.; Kumar, T. T. A. and Prakash, S. (2016). Opportunistic pathogen *Klebsiella pneumoniae* isolated from Maldive's clown fish *Amphiprion nigripes* with hemorrhages at Agatti Island, Lakshadweep archipelago. Int. J. Fish. Aquat., 4(3): 464-467.

Hannan, M. A.; Rahman, M.M.; Mondal, M.N.; Deb, S.C.; Chowdhury, G. and Islam, M.T. (2019). Molecular identification of *Vibrio alginolyticus* causing vibriosis in shrimp and its herbal remedy. Pol. J. Microbiol., 68(4): 429-438.https://dx.doi.org/10.33073%2Fpjm-2019-042

Hansen, G. H.; Raa, J. and Olafsen, J. A. (1990). Isolation of *Enterobacter agglomerans* from dolphin fish, *Coryphaena hippurus* L. J. Fish Dis., 13(1): 93-96.

Haque, E.; Kamruzzaman, M.; Islam, M. S.; Sarwar, T.; Rahman, S. S. and Karim, M. R. (2013). Assessment and comparison of quality of solar tunnel dried bombay duck and silver pomfret with traditional sun dried samples. IJFNEH, 2(4): 187-195.

Haque, A.F.; Khaleque, M.; Deeba, I.M. and Rahman, M.M. (2015). Multidrug-resistant *Rahnella* spp. and *Leclercia adecarboxylata* reservoirs in lakes and rivers of dhaka city: an over looked public health threats, presented at public health foundation day and scientific seminar, ICDDRB, Dhaka, Bangladesh.

Haque, E. (2004). BangladesherChhotoMachh (A book on small fishes of Bangladesh). *Published by Graphic Sign, Mymensingh, 2200*, 81-84.

Hess, B.; Burchett, A. and Huntington, M. K. (2008). *Leclercia adecarboxylata* in an immunocompetent patient. J. Med. Microbiol., 57(7): 896-898.

Hussain, M. M.; Karim, A.; Alam, Z.; Islam, M. S.; Khan, A. and Hossain, A. (1992). Effect of pre-treatment on the drying of ghonia fish *Labeo gonius* in the open sun and in a solar tent. Bangladesh J. Zool., 20(2): 231-238.

International Association of Microbiological Societies. International Commission on Microbiological Specifications for Foods and Roberts, T. A. (1986). *Microorganisms in Foods: Sampling for Microbiological Analysis, Principles and Specific Applications*. Blackie Acad. & Professional.

Immaculate, K.; Sinduja, P.; Velammal, A. and Patterson, J. (2013). Quality and shelf life status of salted and sun dried fishes of Tuticorin fishing villages in different seasons. Int. Food Res. J., 20(4):1855-1859.

Imtiaz, M.M.; Haque, S.A.; Sarker, M.R.; Mufty, M.M.; Ali, M.M.; Parvez, M.S.; Reza, M.S.; Bhuyan, M.S. and Rahman, M.A. (2017). Quality assessment of improved sun dried ribbon fish (*Trichiurus haumela*) by salt and turmeric powder treatment.

Interaminense, J.A.; Nascimento, D.C.; Ventura, R.F.; Batista, J.E.; Souza, M.M.; Hazin, F.H.; Pontes-Filho, N.T. and Lima-Filho, J.V. (2010). Recovery and screening for antibiotic susceptibility of potential bacterial pathogens from the oral cavity of shark species involved in attacks on humans in Recife, Brazili. J. Med. Microbiol., 59: 941–947.http://dx.doi.org/10 .1099/jmm.0.020453-0.

Islam, M. S.; Akteruzzaman, M. and Ahmed, N. (2006). Study on marketing and value chain of some commercially important coastal and marine aquatic products of Bangladesh. Research Report, *Bangladesh Fisheries Research Forum*, Dhaka, Bangladesh.

Kaisar M.A.; Rasul, M.G.; Mansur, M.A.; Khan, M.; Majumdar, B.C. and Hasan, M.M. (2017). Quality aspect and heavy metal content of dry salted hilsa (*Tenualosa ilisha*) of Bangladesh. Int. Res. J. Biol. Sci., 6(2): 16-21.

Kakati, B. K. and Goswami, U. C. (2013). Characterization of the traditional fermented fish product Shidol of Northeast India prepared from *Puntius sophore* and *Setipinna phasa*.

Kakati, B. K. and Goswami, U. C. (2017). Nutritional, microbial and sensory quality evaluation of fermented *Setipinna phasa*, Hamilton 1822, (Phassya Shidal), marketed in North-east India. J. Appl. Nat. Sci., 9(1): 237-244.

Kalaimani, N.; Gopakumar, K. and Unnikrishnan Nair, T. S. (1988). Quality characteristics of cured fish of commerce. Fishery Technology, 25(1): 54-57.

Keren, Y.; Keshet, D.; Eidelman, M.; Geffen, Y.; Raz-Pasteur, A. and Hussein, K. (2014). Is *Leclercia adecarboxylata* a new and unfamiliar marine pathogen?. J. Clin. Microbiol, 52(5): 1775-1776.

Kumar, K.; Prasad, P.; Raman, R. P.; Kumar, S. and Purushothaman, C. S. (2013). Association of *Enterobacter cloacae* in the mortality of *Pangasianodon hypophthalmus* (Sauvage, 1878) reared in culture pond in Bhimavaram, Andhra Pradesh, India. Indian J. Fish., 60(3): 147-149.

Kumar, V.; Sun, P.; Vamathevan, J.; Li, Y.; Ingraham, K.; Palmer, L.; Huang, J. and Brown, J.R. (2011). Comparative genomics of *Klebsiella pneumoniae* strains with different antibiotic resistance profiles. AAC, 55(9): 4267-4276.

LaBauve, A. E. and Wargo, M. J. (2012). Growth and laboratory maintenance of *Pseudomonas aeruginosa*. Curr. Protoc. Microbiol., 25(1): 6E-1.

Lee, S.C.; Fung, C.P.; Liu, P.Y.F.; Wang, T.C.; See, L.C.; Lee, N.; Chen, S.C. and Shieh, W.B. (1999). Nosocomial infections with ceftazidime-resistant Pseudomonas aeruginosa: risk factors and outcome. ICHE, 20(3): 205-207.

Lin, C. H.; Huang, H. T.; Chien, C. C.; Tzeng, D. S. and Lung, F. W. (2008). Purple urine bag syndrome in nursing homes: ten elderly case reports and a literature review. Clin. Interv. Aging, 3(4): 729.

Mahadevan, S.; Dhandapani, B.; Sivaprakasam, S. and Mandal, A. B. (2010). Batch kinetic studies on growth of salt tolerant Pseudomonas aeruginosa secreting protease in a bio-calorimeter. BBE., 15(4): 670-675.

Mahanta, P. and Muzaddadi, A. U. (2012). Post-fermentation preservation of shidal-a fermented fish product of North-East India. Fish. Tech., 49(2): 177.

Majumdar, B.C.; Afrin,F.; Rasul, M.G.; Khan, M. and Shah, A.K.M. A. (2017a). Comparative study of physico-chemical, microbiological and sensory aspects of some sun dried fishes in Bangladesh. Braz. J. Biol., 4(8): 323-331

Majumdar, B. C. and Rashid, I. (2017). Microbiological properties of dry salted Hilsa, *Tenualosa ilisha* (Hamilton, 1822) fish of Bangladesh. J. fish. life Sci., 2(2): 4-9.

Majumdar, R.K. and Basu, S. (2010). Characterization of the traditional fermented fish product Lonailish of Northeast India. IJTK, 9: 453-458.

Majumdar, R. K.; Basu, S. and Prasad, S. V. (2004). Evaluation of biochemical characteristics of a traditional salt fermented fish product of northeast India with special reference to its flavour components. JIFA, 31: 167-176.

Majumdar, R. K.; Basu, S. and Nayak, B. B. (2009). Assessment of nutritional quality of' Shidal'-a fermented fish product of northeast India. JIFA., 36: 25-34.

Maturin, L. and Peeler, J. T. (2001). Laboratory Methods - BAM: Aerobic Plate Count. US Food and Drug Administration.

Mokracka, J.; Koczura, R.; Pawłowski, K. and Kaznowski, A. (2011). Resistance patterns and integron cassette arrays of Enterobacter cloacae complex strains of human origin. J. Med. Microbiol., 60(6): 737-743.

Mukit, S.S.; Sazedul, H.; Roy, S.; Rahman, S and Chakma, S. (2016). Effects of different types and concentrations of salt on the quality aspects of salted hilsa (*Tenualosa ilisha*). IJIR., 1(1):30–39.

Murray, P. R.; Holmes, B.; and Aucken, H. M. (2010). *Citrobacter, Enterobacter, Klebsiella, Plesiomonas, Serratia,* and other members of the enterobacteriaceae. Topley & Wilson's Microbiology and Microbial Infections.

Nahar, S.; Sayeed, M. A.; Alam, M. T.; Majumdar, B. C.; Begum, K. and Rasul, M. G. (2017). Nutritional and microbiological quality of chepa shutki from haor areas of Bangladesh. JNHFS, 5: 1-7. http://dx.doi.org/10.15226/jnhfs.2017.001107

Nayeem, M. A.; Pervin, K.; Reza, M. S.; Khan, M. N. A.; Islam, M. N. and Kamal, M. (2010). Quality assessment of traditional semi fermented fishery product (Chepa Shutki) of Bangladesh collected from the value chain. Bangladesh Res. Pub. J., 4(1): 41-46.

Neto, J. R.; Yano, T.; Beriam, L. O. S.; Destéfano, S. A. L.; Oliveira, V. M. and Rosato, Y. B. (2003). Comparative RFLP-ITS analysis between *Enterobacter cloacae* strains isolated from plants and clinical origin. Arquivos do Instituto Biológico, 70: 367-372.

Norhana, M. W.; Poole, S. E.; Deeth, H. C. and Dykes, G. A. (2010). Prevalence, persistence and control of *Salmonella* and *Listeria* in shrimp and shrimp products: A review. Food Control, 21(4): 343-361.

Nowsad, A. K. M. (2007). *Participatory training of trainers: a new approach applied in fish processing*. BFRF.Freezing of fish 139-140 pp.

Nur, I. T.; Ghosh, B. K. and Acharjee, M. (2020). Comparative microbiological analysis of raw fishes and sun-dried fishes collected from the Kawran bazaar in Dhaka city, Bangladesh. Food Res., 4(3): 846-851.

Oliveira, R.V.; Peixoto, P.G.; Ribeiro, D.D.C.; Araujo, M.C.; do Santos C.T.B.; Hayashi, C.; Pedreira, M.M. and Pelli, A. (2014). *Klebsiella pneumoniae* as a main cause of infection in Nishikigoi *Cyprinus carpio* (carp) by inadequate handling. Braz. J. Vet. Pathol., 7(2): 86-88.

Padua, S.B.; Marques, D.P.; Sebastiao, Fabiana, F.A.; Pilarski, Martins, M.L. and Ishikawa, M.M. (2004). Isolation, characterization and pathology of *Citrobacter freundii* infection in native Brazilian catfish *Pseudoplatystoma.* Braz. J. Vet. Pathol, 7(3): 151 – 157.

Paterson, D.L.; Rossi, F.; Baquero, F.; Hsueh, P.R.; Woods, G.L.; Satishchandran, V.; Snyder, T.A.; Harvey, C.M.; Teppler, H.; DiNubile, M.J. and Chow, J.W. (2005). In vitro susceptibilities of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections worldwide: the 2003 study for monitoring antimicrobial resistance trends (SMART). J. Antimicrob. Chemother., 55(6): 965-973.

Rasul, M.G.; Faruk, M.O.; Sarkar, M.S.I.; Yuan, C.; Akter, T. and Shah, A.K.M.A. (2022_a). Post-mortem biochemical, microbiological and sensory quality changes in the grass carp (*Ctenopharyngodon idella*) during ice storage. Egypt. J. Aquat. Biol. Fish, 26(5): 175 -187.

Rasul, M.G.; Yuan, C.; Yu, K.; Takaki, K. and Shah, A.K.M.A. (2022_b). Factors influencing the nutritional composition, quality and safety of dried fishery products. Food Res., 6(5): 444-466.

Rasul, M.G.; Chunhong, Y. and Shah, A.K.M.A. (2020). Chemical and microbiological hazards of dried fishes in Bangladesh: A food safety concern. Food and Nutri. Sci., 11(6): 523-539.

Reza M. S.; Bapary, M. A. J.; Azimuddin, K .M.; Nurullah, M. and Kamal, M. (2005). Studies on the traditional drying activities of commercially important marine fishes of Bangladesh. PJBS, 8: 1303-1310.

Rohomania, T.; Saha, M. L.; Hossain, A.; Mandal, S. C. and Rahman, M. S. (2014). Microbial and proximate composition of fresh and salted Hilsa, *Tenualosa ilisha*. Bangladesh J. Zool., 42(2): 227-236.

Sadikot, R. T.; Blackwell, T. S.; Christman, J. W. and Prince, A. S. (2005). Pathogen–host interactions in *Pseudomonas aeruginosa* pneumonia. Am. J. Respir. Crit. Care Med., 171(11): 1209-1223.

Sant'Ana, A. (2012). Introduction to the special issue: *Salmonella* in foods: evolution, strategies and challenges. Food Res. Int,, 2(45): 451-454.

Shamim, M. A. H.; Ahmed, M. K.; and Abdullah, A. T. M. (2011). Proximate composition of different portion of hilsa, *Tenualosa ilisha* from two regions of the Bay of Bengal in Bangladesh. Dhaka Univ. J. Biol. Sci., 20(2): 109-115.

Shinkafi, S. A. and Ukwaja, V. C. (2010). Bacteria Associated with Fresh Tilapia Fish (*Oreochromis niloticus*) Sold at Sokoto Central Market in Sokoto, Nigeria. NJBAS, 18(2).

Söhngen, C.; Podstawka, A.; Bunk, B.; Gleim, D.; Vetcininova, A.; Reimer, L.C.; Ebeling, C.; Pendarovski, C. and Overmann, J. (2016). Bac Dive–the bacterial diversity metadata base in 2016. Nucleic Acids Research, 44(D1): D581-D585.

Stock, I.; Burak, S. and Wiedemann, B. (2004). Natural antimicrobial susceptibility patterns and biochemical profiles of *Leclercia adecarboxylata* strains. CMI, 10(8): 724-733.

Storti, A.; Pizzolitto, A. C. and Pizzolitto, E. L. (2005). Detection of mixed microbial biofilms on central venous catheters removed from intensive care unit patients. Braz. J. Microbiol., 36(3): 275-280.

Sultana, N.; Hossain, M. T.; Siddique, M. P.; Uddin, M. I.; Dina, M. A. and Farhana, Z. (2010). Microbial quality of dried fish of different areas Chittagong and Mymensingh districts of Bangladesh. Int. J. Bio. Res., 2(8): 1-5.

Tabassum, K. S.; Islam, M. T.; Chakraborty, S. C. and Rana, M. M. (2018). Assessment of shelf life and quality aspect of market salted and laboratory prepared salted hilsha (*Tenualosa ilisha*). Res. agric., livest. fish., 5(2): 249-257.

Tam, V. and Nayak, S. (2012). Isolation of *Leclercia adecarboxylata* from a wound infection after exposure to hurricane-related floodwater. Case Reports, 2012, bcr-2012.

Tamura, K.; Sakazaki, R.; Kosako, Y. and Yoshizaki, E. (1986). *Leclercia adecarboxylata* Gen. Nov., Comb. Nov., formerly known as *Escherichia adecarboxylata*. Curr. Microbiol., 13(4): 179-184.

Taylor, S. L.; Guthertz, L. S.; Leatherwood, M. and Lieber, E. R. (1979). Histamine production by *Klebsiella pneumoniae* and an incident of scombroid fish poisoning. Appl. Environ. Microbiol., 37(2): 274-278.

ThillaiSekar, V.; Santiago, T.C.; Vijayan, K.K.; Alavandi, S.V.; Stalin Raj, V.; Rajan, J.J.S.; Sanjuktha, M. and Kalaimani, N. (2008). Involvement of *Enterobacter cloacae* in the mortality of the fish, Mugilcephalus. Lett. Appl. Microbiol., 46(6): 667-672.

Troast, J. L. (1975). Antibodies against enteric bacteria in brown bullhead catfish (*Ictalurus nebulosus*, LeSueur) inhabiting contaminated waters. Appl. Microbiol., 30(2): 189-192.

Tsuji, A.; Kaneko, Y.; Takahashi, K.; Ogawa, M. and Goto, S. (1982). The effects of temperature and pH on the growth of eight enteric and nine glucose non-fermenting species of gram-negative rods. Microbiol. Immunol., 26(1): 15-24.

Wang, J.; Chang, S.; Chen, Y. and Luh, K. (2000). Comparison of antimicrobial susceptibility of *Citrobacter freundii* isolates in two different time periods. J Microbiol Immunol Infect, 33(4): 258-262.

Wang, P. C.; Mori, T.; Komori, K.; Sasatsu, M.; Toda, K. and Ohtake, H. (1989). Isolation and characterization of an *Enterobacter cloacae* strain that reduces hexavalent chromium under anaerobic conditions. Appl. Environ. Microbiol., 55(7): 1665-1669.