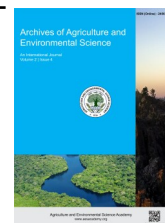




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## ORIGINAL RESEARCH ARTICLE



## Evaluation of microbiological quality of dried baim (*Mastacembelus armatus*) in Bangladesh

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### ABSTRACT

A study was performed for five months to assess the microbiological quality of dried Baim (*Mastacembelus armatus*) in Sylhet region of Bangladesh. A total of 45 dried samples were randomly collected on monthly basis from three different sources of Sylhet region; one from producer (drying yard of Lamagaji) and others from retail market (Bandar Bazar) and control (prepared in laboratory). The mean total plate count (TPC) of dried Baim from producer, retail market and control were observed  $6.20 \pm 0.72 \times 10^5$ ,  $9.64 \pm 1.58 \times 10^5$  and  $1.61 \pm 1.06 \times 10^5$  cfu/g, respectively, whereas, average total fungal count (TFC) were estimated  $3.77 \pm 0.81 \times 10^3$ ,  $4.65 \pm 1.08 \times 10^3$  and  $1.78 \pm 0.64 \times 10^3$  cfu/g, respectively. TPC and TFC of dried Baim of retail market were found significantly ( $P < 0.05$ ) highest and significantly ( $P < 0.05$ ) lowest in control samples than others. Twenty five samples from each source were analyzed to determine pathogenic *E. coli* and *Salmonella* sp. Hundred percent samples of dried Baim of producer and retail market were found contaminated by *E. coli* whereas; the controlled samples were free of *E. coli*. Likewise, the dried Baim samples of producer and retail market were contaminated 60% and 80% respectively with *Salmonella*. Dried baim sample from market was 100%, producer sample was 62% and control sample was 32% contaminated with fungi. The most common fungus species in samples were *Aspergillus fumigatus*, *Fuserium proliferatum* and *Rhizopus stolonifer*. The overall microbiological quality of control samples was comparatively better than the commercially produced dried Baim in Sylhet region.

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### INTRODUCTION

Bangladesh has the world's biggest flooded aquatic wetland after China and India; it is the third largest aquatic biodiversity in Asia. The contribution of the fisheries sector is 3.69 per cent in the Gross Domestic Product (GDP) of the country and 22.60% to the agricultural GDP (FRSS, 2016). In the FY 2017-2018, Bangladesh earned Tk 4,500 crore by exporting at least 69,000 tonnes of fish and fish products. The greater Sylhet region of Bangladesh together with Moulovibazar, Sunamganj and Habiganj has vast water bodies and is enriched with *haor* fisheries

(Mazid and Kamal, 2005). During winter season, huge quantities of small fishes are usually harvested from these areas than those of the other seasons.

Drying is a food preservation method that works by lowering water activity from the food, which prevents the growth of microbes (Bala and Mondol, 2001; Bellagha et al., 2002; Duan et al., 2004; Beuchat et al., 2013). Open air sun drying has been practiced from ancient times to preserve food for longer period of time (Rasul et al., 2018; Hasan et al., 2016). The proportion about 20% of the artisanal catch is being dried by traditional sun drying methods and consumed in the domestic market (Nowsad,

2007). The quantity of dry fish export would raise by 0.46% yearly. The total amount would increase by 2.30% i.e., from 355.30 thousand tones to 363.30 thousand tones during the period from 2010-11 to 2015-16 in Bangladesh (Sen et al., 2015). Though, dried fish is a low cost source of high quality protein to the low income population (Petrus et al., 2013), the consumers now a day are very much concerned about the quality of dried products, particularly chemical contaminants, spoilage and infestation by blow flies. Traditional drying is often undeveloped and good hygiene and sanitation is rarely practiced. Various microorganisms adversely affect to the quality of sun dried fishes. It has been stated that dried food and their ingredients were contaminated by pathogenic and harmful microorganisms like as *Escherichia coli* O157:H7 (Deng et al., 1998), and *Salmonella* sp. (Archer et al., 1998; Beuchat and Mann, 2011). The incidence of *Salmonella* spp. in various dried fishes directly indicated the maintenance of low as well as poor hygienic condition during dried fish processing (Sultana et al., 2010). Particularly, some pathogenic molds have been found as considerable amount in dried food (Hyun et al., 2018). There are some species of spoilage molds and osmophilic yeasts that can grow at relatively low Aw values from 0.60 to 0.70 (Cousin et al., 2005). The presence of the pathogenic microbial loads in dried fishes is getting importance in the safety and quality aspects of the dried fishes (Patterson and Ranjitha, 2009). So, is very essential to determine the microbiological quality of such processed fishes for guarding consumer's health and hygiene (Lilabati et al., 1999). The information about the microbial load and pathogenic bacteria of dried Baim associated with fungal strain in dried fish products is lacking which drive us to look into it. Detailed microbial qualities make sure that products available to consumers are not only nutritious but also free of potentially harmful microbiota (PHM). So, it is very important to assess microbiological quality and safety of dried fishes in retail trade for protecting health and hygiene of local consumer. The present study thereby aimed and conducted to determine microbiological quality of dried Baim of Sylhet region.

## MATERIALS AND METHODS

### Collection of samples

Dried Baim (*Mastacembelus armatus*) was selected as sample dried fish which is one of the most available and popular dry fish in Sylhet region of Bangladesh. Sample was collected from three different sources on monthly basis; Lamagaji fish drying yard of Sunamgonj, Banadar Bazar (Retail Market) of Sylhet Sadar and control sample prepared in every month of study period in laboratory of Department of Fisheries Technology and Quality Control, SAU with proper hygienic and sanitation condition. Raw fresh Baim used to prepare control sample were collected from Tilagor fish market, Sylhet Sadar uazilla of Sylhet district. The dried fish was packed into airtight polyethene bags and stored at room temperature for subsequent analysis. Samples were collected as monthly basis from December 2016 to April 2017.

### Total plate count (TPC)

Bacterial load was determined using plate count agar (PCA) by spread plate technique. Ten grams of the sample was mixed with 10 fold volume of physiological saline (0.85% NaCl) which was serially mixed ten folds. Appropriate dilutions of fish homogenate were spread on plate count agar. Then incubated at 37°C for 24 hours and the colonies were counted for total plate count having plates 30-300 colonies and the count was expressed as cfu/g (APHA, 1992).

### Total fungal count (TFC)

Fungal count was conducted out using sabouraud dextrose agar (SDA) to which Chloramphenicol (antibiotics) was mixed. Twenty-five grams of the sample was blended with 225 ml of 0.05% agar in saline solution (0.85% NaCl) and 0.1 ml of the appropriate dilutions of the sample was spread on the surface of the medium. Then incubated at room temperature (28±1°C) for 3-5 days and the colonies were counted for total fungal count and the count was expressed as cfu/g (Yamagata, 1992).

### Detection of pathogenic bacteria *E. coli*

Homogenated dry fish was transferred to LSTB tubes. Then incubated at 37°C for 24 hours and observed for growth and gas production. Samples from positive LSTB tubes were transferred to EC broth tubes and incubated at 37°C for 24-48 hours. Samples from positive EC broth was streaked on to eosin methylene blue (EMB) agar plate to sure that the *E. coli*. Black or dark centered colonies with or without greenish metallic sheen were produced by *E. coli*. Further biochemical tests (Sugar fermentation test, Indole test, MR test, VP test, TSI slant test and Catalase test) were completed for identification of *E. coli* (AOAC, 1998).

### *Salmonella* spp.

In the detection of *Salmonella*, lactose broth (LB) was used which act as pre-enrichment and tetrathionate broth and selenite cystine broth were used in enrichment. Isolation for *Salmonella*, xylose lysine deoxycholate (XLD) was used. Further biochemical tests were done for identification of *Salmonella* (AOAC, 1998). *Salmonella* exhibited pink colonies with or without black centers (FDA BAM, 2007).

### Isolation of fungal organisms

At first, ten-fold serial dilution of 1g of fish with distilled water then 0.1ml of the dilution was cultured by spread plate technique into Potato dextrose agar (PDA) supplemented with chloramphenicol at 40 µg/ml and Gentamycin at 500 µg/ml. Then incubated for 5 to 14 days at room temperature. Pure culture of the different colonies (based on morphology) was obtained by sub-culture of the isolates on potato dextrose agar plates and sabouraud's dextrose agar plates. The fungal isolates were identified to the genus/species level based on macroscopic and microscopic characteristics and all of the isolates obtained from pure cultures.

### Culture of samples

From the sample an inoculum is prepared and streaked on the SDA media and incubated at room temperature. The different plate media were looked for the fungal growth every day until growth is found after 3 to 4 days of plating. Pure culture was prepared from this initial culture (Rippon, 1998).

### Preparation of pure culture

In order to make a pure culture, spores from initial culture was transferred to media containing petridishes by sterilized inoculating loop to avoid any contamination with other fungus and incubate for 3-4 days at room temperature until the fungal growth is found.

### Identification of fungus

Fungal colony was taken with the help of an inoculating needle on a fresh glass slide containing two drops of lactophenol cotton blue from pure culture. The fungal colony was covered with a cover slip and the slides were examined under the microscope. The fungus was identified on the basis of its cultural and morphological characteristics (Rippon, 1998).

### Data analysis

All data were subjected to statistical analysis using one-way ANOVA including IBM SPSS, Version 20.0. Probabilities of  $P < 0.05$  were considered significant. Significance differences between means were assessed using the Duncan's multiple range test.

## RESULTS AND DISCUSSION

### Quantitative microbiological analysis of dried Baim fish

#### Total plate count (TPC) of dried Baim fish

Results of total plate count of dried Baim fishes from three different sources are presented in Table 1. In each month significantly ( $P < 0.05$ ), the highest bacterial load was found in Market

source compared to control source. In the month of February and March, no significant differences were observed between bacterial load of Producer and Market source. Similarly, no significant difference was found in the bacterial of Producer and Control source in the month of February. Overall, significantly ( $P < 0.05$ ) the highest and the lowest bacterial count were observed in the month of April and January, respectively.

#### Total fungal count (TFC) of dried Baim fish

Results of total fungal count of dried Baim fishes collected from different sources are shown in Table 2. In each month, significantly ( $P < 0.05$ ) the lowest fungal count were estimated in Control source. Significantly the highest fungal count was found in Market source in the month of December, March and April. Whereas, significantly the highest fungal load was observed in Producer source in the month of February and April. However, no significant differences were found between the fungal counts of Producer and Market source in the month of January, February and April. Likewise, no significant differences were found between the fungal counts of Producer and Control source. Overall, significantly ( $P < 0.05$ ) the highest and the lowest fungal counts were observed in the month of March and January, respectively.

### Isolation and identification of indicator bacteria (*Escherichia coli*)

#### Cultural properties of *E. coli*

*E. coli* is an aerobic and facultative anaerobic bacteria. Optimum temperature for growth was 36-37°C where most strains grew over the range of 18- 44°C. *E. coli* is a Gram negative rod shaped bacteria and it varies from coccoid bipolar shape to long filamentous form. The colonies usually developed to a size of 2-3 mm on agar media. *E. coli* found metallic sheen on the EMB agar, also found rose pink colony on the MacConkey agar and pinkish colony on the *Salmonella-Shigella* (SS) agar medium (Table 3).

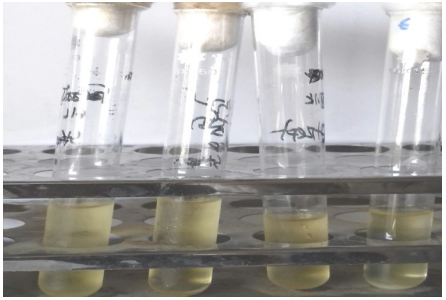
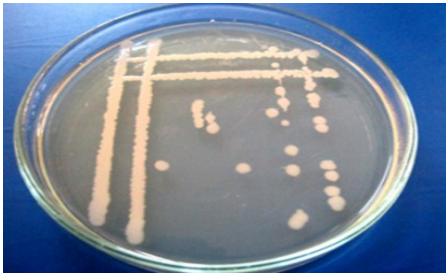
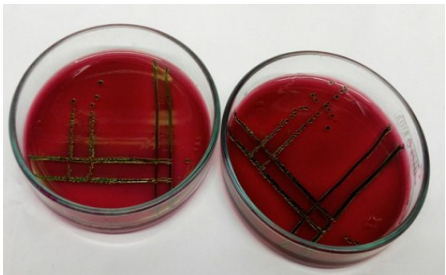

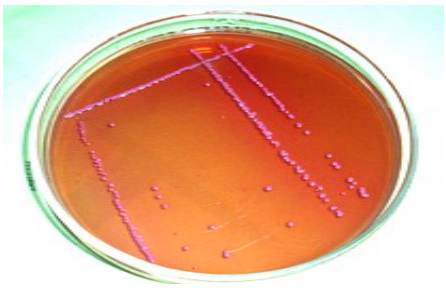
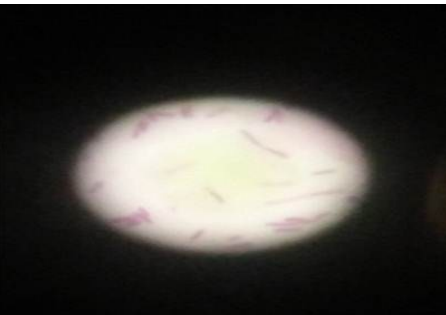
**Table 1.** Total plate count (cfu/g) (Mean  $\pm$  SD) of dried Baim fish from different sources (n=3). Different superscripts in each row represent significant difference ( $P < 0.05$ ).

Month	Sample source		
	Producer	Market	Control
December	6.70 <sup>b</sup> $\pm$ 0.40 $\times$ 10 <sup>5</sup>	9.77 <sup>c</sup> $\pm$ 0.95 $\times$ 10 <sup>5</sup>	1.20 <sup>a</sup> $\pm$ 0.30 $\times$ 10 <sup>5</sup>
January	5.60 <sup>b</sup> $\pm$ 0.30 $\times$ 10 <sup>5</sup>	8.23 <sup>c</sup> $\pm$ 0.55 $\times$ 10 <sup>5</sup>	0.93 <sup>a</sup> $\pm$ 0.25 $\times$ 10 <sup>5</sup>
February	6.03 <sup>b</sup> $\pm$ 0.15 $\times$ 10 <sup>5</sup>	8.90 <sup>c</sup> $\pm$ 0.90 $\times$ 10 <sup>5</sup>	1.03 <sup>a</sup> $\pm$ 0.55 $\times$ 10 <sup>5</sup>
March	5.55 <sup>b</sup> $\pm$ 0.55 $\times$ 10 <sup>5</sup>	9.00 <sup>c</sup> $\pm$ 0.10 $\times$ 10 <sup>5</sup>	1.40 <sup>a</sup> $\pm$ 0.40 $\times$ 10 <sup>5</sup>
April	7.13 <sup>b</sup> $\pm$ 0.41 $\times$ 10 <sup>5</sup>	12.30 <sup>c</sup> $\pm$ 0.62 $\times$ 10 <sup>5</sup>	3.50 <sup>a</sup> $\pm$ 0.70 $\times$ 10 <sup>5</sup>

**Table 2.** Total fungal load (cfu/g) (Mean  $\pm$  SD) of dried Baim fishes from different sources (n=3). Different superscripts in each row represent significant difference ( $P < 0.05$ ).

Month	Sample source		
	Producer	Market	Control
December	3.57 <sup>b</sup> $\pm$ 0.75 $\times$ 10 <sup>3</sup>	5.20 <sup>c</sup> $\pm$ 1.05 $\times$ 10 <sup>3</sup>	1.40 <sup>a</sup> $\pm$ 0.40 $\times$ 10 <sup>3</sup>
January	4.14 <sup>b</sup> $\pm$ 0.95 $\times$ 10 <sup>3</sup>	3.50 <sup>b</sup> $\pm$ 0.70 $\times$ 10 <sup>3</sup>	2.10 <sup>a</sup> $\pm$ 0.65 $\times$ 10 <sup>3</sup>
February	3.07 <sup>b</sup> $\pm$ 0.20 $\times$ 10 <sup>3</sup>	4.50 <sup>c</sup> $\pm$ 0.90 $\times$ 10 <sup>3</sup>	1.50 <sup>a</sup> $\pm$ 0.70 $\times$ 10 <sup>3</sup>
March	3.83 <sup>b</sup> $\pm$ 1.05 $\times$ 10 <sup>3</sup>	5.23 <sup>c</sup> $\pm$ 1.05 $\times$ 10 <sup>3</sup>	2.30 <sup>a</sup> $\pm$ 0.65 $\times$ 10 <sup>3</sup>
April	4.26 <sup>b</sup> $\pm$ 0.80 $\times$ 10 <sup>3</sup>	4.83 <sup>c</sup> $\pm$ 1.30 $\times$ 10 <sup>3</sup>	1.60 <sup>a</sup> $\pm$ 0.70 $\times$ 10 <sup>3</sup>

**Table 3.** Cultural characteristics of isolated *E. coli* on different media.

Feature	Appearance	Pictorial view
Nutrient broth	Turbidity, cloudiness in the broth, heavy sediment at the bottom of the test tube	
Nutrient Agar	Smooth, circular, white to grayish white colonies were found.	
Eosin Methylene Blue Agar (EMB)	Smooth, circular, black color colonies with metallic sheen was produced.	
MacConkey Agar (MC)	Rose pink lactose fermented colonies were formed.	
Salmonella- Shigella Agar	Slight pinkish colonies	
Gram's staining	<i>E. coli</i> is a gram negative rod shaped	

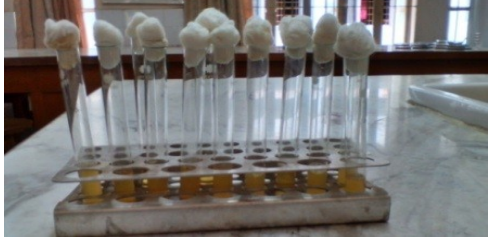

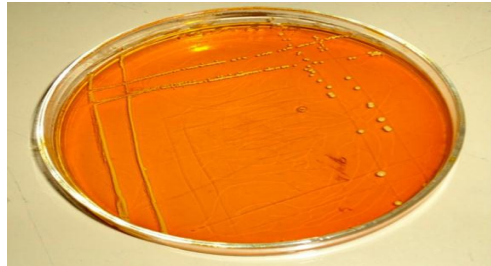
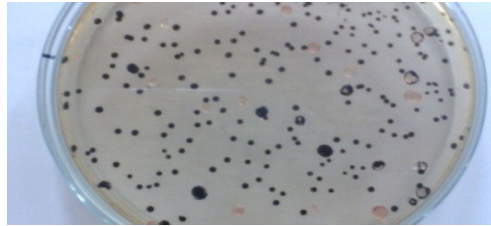

### Biochemical tests

All of the *E. coli* isolates fermented all five basic sugars like dextrose, maltose, lactose, sucrose and mannitol with the production of acid. Acid production was reported by the color change from reddish to yellow and gas production was marked by the presence of gas bubbles in the inverted Durham's tubes. On TSI agar slant, *E. coli* showed positive result by indicating slant red and butt yellow colored. *E. coli* gives negative reaction to Voges Proskauer (VP) test and positive reaction to Methyl red test (MR test) which was indicated by stable red color. *Escherichia coli* produce vigorous gas bubble which was indicative of Catalase positive and negative to VP reaction test (Table 4).

**Table 4.** Results of different biochemical tests.

Indole	MR	VP	Catalase test	TSI Slant	Sugar fermentation test			
					Dextrose	Maltose	Mannitol	Lactose
+ve	+ve	-ve	+ve with gas bubble	yellow	+ve	+ve	+ve	-ve

**Table 5.** Cultural characteristics of *Salmonella* in different culture media.

Media used	Cultural characteristics	Pictorial view
Nutrient broth	Produced turbidity	
Nutrient agar	Translucent, opaque, smooth, circular colonies	
MacConkey agar	Grow colorless colonies	
<i>Salmonella</i> agar	Black centered colonies	
Gram's staining method	Gram-negative, pink colored	

### Isolation and identification of pathogenic bacteria, *Salmonella*

#### Cultural properties of *Salmonella*

*Salmonella* is Enterobacteriaceae which is gram negative and motile with peritrichous flagella. *Salmonella* is non spore forming rods and the rods are typically 0.7-1.5µm×2.5µm in size. *Salmonella* is facultative anaerobic which can grow with or without oxygen. *Salmonella* show catalase positive and oxidase negative reactions. *Salmonella* produce turbidity on the NB agar, colorless colony on the MacConkey agar and black centered colony on the Salmonella-Shigella (SS) agar medium (Table 5).

### Biochemical tests

All of the *Salmonella* isolates fermented three sugars dextrose, maltose and mannitol with the production of acid and gas but did not ferment lactose and sucrose. Acid production was noted by the color change from reddish to yellow and gas production was marked by the presence of gas bubbles in the inverted Durham's tubes. On TSI agar slant, *Salmonella* sp. showed positive result by indicating black colored butt. *Salmonella* sp. gives positive reaction to Methyl red test (MR test) which was indicated by stable red color and negative reaction to Voges Proskauer (VP) test. *Salmonella* sp. gives negative reaction to indole test and produce gas bubbles which were indicative of Catalase test positive (Table 6).

### Isolation of *E. coli* and *Salmonella* from dried Baim fishes

In each month, three dried Baim fish samples from each source were analyzed to determine the qualitative microbiological quality. In total, 25 dried Baim fish samples from each source were analyzed. *E. coli* was isolated from all dried Baim fish samples of Producer and Market source in all five months. But in case of control dried Baim fish sample, *E. coli* was found in the sample of the month of December and March. *Salmonella* was observed in the dried Baim fish sample of Producer in the month of March and April. Whilst, samples of Market source were found *Salmonella* positive in the month of December, March and April. However, all dried Baim samples of control was found free from *Salmonella* contamination (Table 7).

All the dried Baim fishes samples from Producer (Lamagaji) and Retail Market (Bandar Bazar) were found contaminated by *E. coli*. 80% dried Baim fishes sample of Market were found *Salmonella* and 60% dried Baim fishes sample Producer were observed contaminated by *Salmonella*. However, control

samples were recorded free from *Salmonella* (Table 7).

### Identification of fungal species

#### *Aspergillus fumigatus*

For the *A. fumigatus* the colony have a diameter of 2-3 cm in 5 days. The flat colonies were white at first, whitish green as conidia began to mature, especially near the center of the colony.

#### Identification of *Fuserium proliferatum*

*Fuserium proliferatum* - Cultural character-Observe-Wooly, Cotton candy like and white and reserve - pink. Microscopic Character-Branched conidia located with conidiophores.

#### Identification of *Rhizopus stolonifer*

*Rhizopus stolonifer* - Cultural character-Observe-Colony is white initially then turns grey to yellowish brown in time and reserve-White to pale. Microscopic Character - non separate or densely separate broad hypae, sporangiophores, rhizoids (root like hypae), sporangia and sporangiospores are exposed.

### Percentage distribution of different fungal species in dried Baim fishes

Of the total 75 dried Baim fish sample resolved for the presence of fungal agents, 48 samples are positive for one or more fungal species. Fungal isolated were found 25 in retailer market, 15 in producer, 8 in control sample (Table 8).

Of the fungal agents diversified from Retail market sample, *Aspergillus* sp. 25 (100%) from total sample 25, has the higher recurrence, producer sample *Aspergillus* sp. 12 (48%) from total sample 25, and control sample *Aspergillus* sp. 7 (21%) from total sample 25 (Table 8).

**Table 6.** Biochemical characteristics of *Salmonella* on different test.

Indole	MR	VP	Catalase test	TSI Slant	Sugar fermentation test			
					Dextrose	Maltose	Mannitol	Lactose
-ve	+ve	-ve	+ve with gas bubble	Red	+ve	+ve	+ve	-ve

**Table 7.** Summary of isolation of *E. coli* and *Salmonella* from dried Baim.

Sample		Isolated bacteria	
		<i>E. coli</i>	<i>Salmonella</i>
Retailer market (Bandar Bazar)	No of sample analysed	25	25
	No of positive sample	25	20
	% of positive sample	100	80
Producer (Lamagaji)	No of sample analysed	25	25
	No of positive sample	25	15
	% of positive sample	100	60
Control	No of sample analysed	25	25
	No of positive sample	0	0
	% of positive sample	0	0
Total	No of sample analysed	75	75
	No of positive sample	50	35
	% of positive sample	66.67	46.67

Of the fungal agents isolated from Retail market sample, *Fuserium* sp. 19 (76%) from total sample 25, has the higher frequency of occurrence, producer sample *Fuserium* sp. 10(40%) from total sample 25, and control sample *Fuserium* sp. 4(16%) from total sample 25 (Table 8).

Of the fungal agents isolated from Retail market sample, of *Rhizopus* sp. 17 (68%) from total sample 25, has the higher frequency of occurrence, producer sample of *Rhizopus* sp. 5 (20%) from total sample 25, and control sample of *Rhizopus* sp. 4 (16%) from total sample 25 (Table 8).

Now a day, food quality and safety is one of the major troubles in the whole world; that forced many researchers to discuss methods of addressing consumer concerns with various aspects of food safety and quality (Nielsen et al., 2009). So, our current study was conducted to perform quantitative and qualitative analysis of microbes in dried Baim.

Mean total bacterial load of the dried fishes of Producer, Market and Control were observed  $6.20 \pm 0.72 \times 10^5$ ,  $9.64 \pm 1.58 \times 10^5$  and  $1.61 \pm 1.06 \times 10^5$  cfu/g, respectively. It has been reported that TPC in sun-dried baim fish samples ranged from  $3.7 \times 10^3$  (0 day) to  $3.3 \times 10^6$  cfu/g (6 month) respectively (Bilgin et al., 2008). Hasan et al. (2006) found that the bacterial load of traditional, rotary and solar tunnel dried products were in the range of  $1.43 \times 10^8$  to  $2.89 \times 10^8$  cfu/g,  $1.91 \times 10^8$  to  $2.84 \times 10^8$  cfu/g and  $1.95 \times 10^8$  to  $2.59 \times 10^8$  cfu/g, respectively. With increase in duration of storage, total viable counts (TVC) of dried fish samples were increased due to growth and multiplication of the microbes (Bilgin et al., 2008). The bacterial count in dried fishes was less than  $10^7$  cfu/g (Sanjeev, 1997). Lilabathi et al. (1999) reported the there is a direct relationship between the total microbial count and water content of the dried fish sample. Patterson and Ranjitha (2009) enumerated that total plate count (TPC) seemed to be high in the commercially dried fishes than the experimentally dried fishes. Our finding is similar with the results reported by Majumdar et al. (2017) Rasul et al. (2018) and Majumdar et al. (2018). Significantly ( $P < 0.05$ ) the highest and the lowest bacterial count were observed in the month of April and January, respectively. This variation in TPC might occur because of the differences in moisture and temperature in different months. In every circumstance, maximum TPC was observed in market sample and minimum TPC was observed in control sample. ICMSF (ICMSF, 1986) suggested that quality levels are raised on the plate counts with representative sample unit less than  $5 \times 10^5$  cfu/g are good quality between  $5 \times 10^5$ – $10^7$  cfu/g marginally accepted quality and plate count at or above  $10^7$  are considered unacceptable in quality and safety.

The quality of dry fishes was adversely influenced by occurrence of fungi (FDA, 1982). Mean fungal load of dried Baim fishes of Producer, Market and Control were estimated  $3.77 \pm 0.81 \times 10^3$ ,  $4.65 \pm 1.08 \times 10^3$  and  $1.78 \pm 0.64 \times 10^3$  cfu/g, respectively. It has been reported that TFC of dried punti were varied from  $1.15 \pm 0.10 \times 10^2$  cfu/g to  $7.43 \pm 0.25 \times 10^4$  cfu/g in producer, retail market and control sample (Hossain et al., 2016). Sulieman and Mustafa (Sulieman and Mustafa, 2012) estimated the fungal load of dried fishes from Eldeim area (Central Sudan) in the range of  $1.5 \times 10^2$  –  $5.3 \times 10^4$  cfu/g. Saritha et al. (2012) reported that the range of TFC were  $1.3 \times 10^4$  –  $2.2 \times 10^4$  cfu/g in dried fishes of Cuddalore district, India.

Seasonal variation in water content of dried fish products could be depend on the result of different drying time, environmental changes and level and type of salt used for curing (Anihouvi et al., 2006). In our study, in all the dried fish seasonal variation in the fungal population was observed. Patterson and Ranjitha (2009) calculated TFC from commercially and experimentally dried fishes exposed that total fungal count seemed to be high in the commercially dried fishes than the experimentally dried fishes. Overall, significantly ( $P < 0.05$ ) the highest and the lowest fungal counts were observed in the month of March and January, respectively. No such significant differences were found between the fungal loads of the month of December, March and April. This observation seems suggest that the variation of TFC in different months because of the differences in moisture and temperature level. Significantly the highest TFC were observed in the dried Baim of Market.

Indicator bacteria i.e. *E. coli* has been isolated and identified from dried Baim of different sources. The colony characteristics of *E. coli* in this study, on EMB agar found smooth, circular, black centred colonies with metallic sheen, on NA found smooth, circular and white colored colonies, also found slight growth of pink to red colonies on SS agar and smooth pinkish colonies on MCA which responds well with observations of several other authors (Sharada et al., 2009; Buxton and Fraser, 1977). In Gram's staining, the morphology of the isolated bacteria developed gram negative short rod arranged in single or paired and in hanging drop slide tests it was found motile which were parallel to the reports of (Sharada et al., 2009; Buxton and Fraser 1977; Merchant and Packer, 1967). The isolates also stated positive reaction in MR test and Indole test but negative reaction in VP test and in TSI slant test it was found yellow slant and butt with gas but no  $H_2S$  production which were similar to the observations of (Buxton and Fraser, 1977).

**Table 8.** Percentage distribution of different fungal species in dried Baim fishes.

Sample source	Total sample	<i>Aspergillus</i> sp.		<i>Fuserium</i> sp.		<i>Rhizopus</i> sp.	
		Presence	Percentage	Presence	Percentage	Presence	Percentage
Market	25	25	100%	19	76%	17	68 %
Producer	25	12	48%	10	40 %	5	20%
Control	25	7	21%	4	16 %	4	16%
Total	75	44	58.67%	33	44%	26	26%

In our present study, 75 samples of dried Baim fishes were analyzed to isolate and identify *E. coli* and 50 samples were found contaminated with *E. coli*. All 25 samples (100%) of Producer and Retail Market were recorded *E. coli*. *E. coli* is endangered because the production of histamine in the dried fishes (Logesh et al., 2012). The presence of faecal coliform bacteria, including *E. coli* in most of the samples examined indicates poor hygiene and sanitary condition. This finding verifies with the study of (Feng et al., 2014).

Cultural characteristics of *Salmonella* were recorded. In Gram's staining, the morphological characteristics of the isolated *Salmonella* exhibited Gram negative, small rod shaped, single or paired in arrangement under microscope which was approved by work of other researchers (Gene, 2002; Jones et al., 1997 and Freeman, 1985). Besides, in sugar fermentation test, all the *Salmonella* isolates fermented dextrose, maltose and mannitol and produce acid and gas but did not ferment sucrose and lactose which is similar to the report of (Buxton and Fraser, 1977). Again, all the *Salmonella* isolates were positive to methyl red test and negative to indole and VP test and in TSI slant test, it was found but remained yellow and slant converted to pink color (Merchant and Packer, 1967). These results are related to the results referred by (Rahman, 1977); Cherry et al., 2004). A number of 75 (25 samples from each source) dried Baim samples were analyzed to isolate and identify *Salmonella*. Overall, 46.67% samples of dried Baim were found contaminated by *Salmonella*. 80%, 60% samples of Producer and Retail Market were recorded *Salmonella*, respectively. No *Salmonella* was found in Control samples of dried Baim.

Different types of fungal species were also isolated in the dried Baim samples. Dried samples from Market was 100% contaminated with fungi, Producer sample was 62% contaminated and Control sample was 32% contaminated with fungi. This fungal community may be due to the variation in the chemical and nutritional composition of the fish species and for that reason, different moulds react differently in various fish (Reed et al. 1967; Fafioye et al., 2002). The various organisms were determined under the light microscope showed *Aspergillus flavus* as yellowish green and *A. niger* as dark in colour. However, these two *Aspergillus* species are microscopically alike. These are the most common fungi invading dried fish species in the study area. Similar results were observed by Doe (Doe, 1983) (Alfred-Ockiya and Akeodi, 1998). However, the fungus *Aspergillus flavus* is mainly accountable for the production of aflatoxin and it also causes food borne intoxication which leads to serious health hazards of consumers (Kumar et al., 2017). So safety procedures should be taken to reduce the contaminations and insect infestations of dried fish.

## Conclusion

The microbiological condition in dried Baim was poor in our study areas which may be caused by the improper and unhygienic handling, lack of sanitation of labours, poor processing, improper storage and inadequate packaging of the products. Therefore, control measures such as ensuring

scientific, developed and hygienic fish drying method (e.g. use of good quality raw material, good quality salt, hygienic handling practices, potable water, good quality packaging material), training of the fisher folks and increasing the awareness of mass people about food safety should be taken.

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**Conflicts of Interest:** The authors declare that they have no conflict of interest.

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