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BACTERIAL FLORA ISOLATED FROM DRIED FISHES SOLD AT RETAIL MARKETS WITHIN DHAKA CITY CORPORATION OF BANGLADESH

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Along with other contaminants, bacterial contamination in the dried fishes is a common issue which severely affects the quality of cured fishes. Hence, the present study was aimed to isolate and identify different bacterial flora contaminating different dried fishes, sold at different retail markets within Dhaka Metropolitan city, Dhaka, Bangladesh. A total of 25 different dried fishes were collected from Dhaka North city corporation area (Town Hall kacha bazar, Krishi market, and Mohammadpur kacha bazar) and Dhaka South city corporation area (New market kacha bazar, and Jatrabari chowrasta bazar). Each sample was divided into three regions (head, body, and tail) and bacterial isolation and identification was done by studying their cultural, staining and biochemical properties. Of the 25 dried fishes, *Escherichia coli*, *Bacillus* spp., *Staphylococcus* spp., *Salmonella* spp. and unidentified bacteria isolation rates were 44%, 56%, 80%, 48%, and 24%, respectively. The presence of *E. coli* and *Salmonella* spp. in various dried fish samples could be considered as indication of lack of hygienic condition during dried fish processing. Confirmation through molecular detection methods, pathogenicity, and antibiogram of the isolated bacteria could be included for future study.

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INTRODUCTION

At present, fish is considered as one of the major sources of animal protein (more than 70%) with high biological value (Mazumder *et al*., 2008). Fish and fishery products ranks third in the export item of Bangladesh (Hossain *et al*., 2015) and has been contributing a significant role in human nutrition, employment opportunities, and foreign currency earning (Rana *et al*., 2020). Beside fresh fishes, the dried fishes, commonly known as '*shutki*' in Bangladesh, are highly preferred by all classes of peoples Bangladesh due to characteristics texture, taste, and flavor (Rana *et al*., 2020). Moreover, on dry weight basis, dried fishes have greater nutritional values than fresh fishes (Rasul *et al*., 2018). In fact, drying of fish is one of the oldest, low-cost, and effective methods of fish preservation technique, not only in Bangladesh, but also in other Asian and African countries (Rasul *et al*., 2020). Drying of fish, in most cases, terminates the enzymatic activity in muscles and hampers microbial growth through reducing the water activity (a_w) of fish (Balachandran, 2001). Ultimately, drying ensures the microbial stability and extended shelf-life of fish and fish-products (Balachandran, 2001).

An investigation on the utilization and marketing distribution of fish revealed 25% of the total fishes, available in markets, are dried fish (Islam, 2006). However, food safety is of prime importance and globally biggest public health issue (Nur *et al*., 2020; Rasul *et al*., 2020). In Bangladesh, sun drying is the traditional and sole method of fish drying which is weather-dependent, i.e., depends upon temperature, humidity, air-velocity (Reza *et al*., 2009). Beside weather-dependency, lack of proper hygiene and sanitation, and necessary facilities during drying, harvesting, storage, and marketing of dried fish (Hasan *et al*., 2016). Moreover, improper packaging and storage, and remaining exposed in the retail market, enhances the growth of microbial population due to absorption of moisture from the humid environment (Mazumder *et al*., 2008; Islam *et al*., 2013). Again, bacterial spores are not destroyed by traditional sun-drying of fish (Hyun *et al*. 2018). Various microbes severely affect the quality of dried fishes at every steps of production to marketing of dried fishes worldwide (Sultana *et al*., 2010), threatening the safety and quality aspects of dried fish (Patterson and Ranjitha, 2009).

There are numerous reports on microbial quality of dried fishes and presence of various pathogenic and/or spoilage bacteria in dried fishes, available in the retail markets of Bangladesh (Sultana *et al*., 2010; Paul *et al*., 2018; Nur *et al*., 2020; Rana *et al*., 2020; Rasul *et al*., 2020). Consequently, it has been strongly recommended that regular monitoring of dried fish, regarding microbial quality and microbial presence should be conducted (Rana *et al*., 2020). Hence, the present study was undertaken with the aim to isolate and identify bacterial flora from dried fishes sold at retail markets within Dhaka City Corporation of Bangladesh.

MATERIALS AND METHODS

Study areas and period

In the present study, common bacterial agents were isolated and identified from dried fish samples, sold at different retail markets within Dhaka City Corporation area, both Dhaka North and South City Corporation areas. The whole research work was conducted in the Bacteriology Laboratory of the Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, during the period from October 2019 to March 2020.

Sample collection and transportation

A total of 25 different dried fish samples were collected from Dhaka North city corporation area (Town Hall kacha bazar, Krishi market, and Mohammadpur kacha bazar) and Dhaka South city corporation area (New market kacha bazar and Jatrabari chowrasta bazar). Among 25 dried fish (*shutki*) samples, Loitta, Surma, Tengra, Putti, and Mola were collected from Town Hall kacha bazar, Batashi, Chela, Baime, Dhela, and Taki from Krishi market, Mohammadpur, and Khoilsha, Chapila, Suri, Guinna, and Bata from Mohammadpur kacha bazar, covering Dhaka North City Corporation area. Similarly, Shoil, Salted ilisha, Loitta, Surma, Tengra were collected from New market kacha bazar and Chingri, Mola, Chapila, Bata, and Puti from Jatrabari chowrasta bazar, covering Dhaka South City Corporation area. The samples were collected aseptically in sterile plastic zipper bag of various sizes separately. Then the collected samples were transported to the laboratory and preserved at 4°C.

Processing of samples

The fish samples were processed according to the method, described by Sultana *et al*. (2010). Briefly, each fish samples were cut into three parts, i.e., head, body (abdominal part), and tail regions. From each portion, an amount of 10 grams of samples were collected and was then minced and grinded properly with 90 ml of 1% peptone water using previously sterilized and dried mortar and pestle. About 5 ml of fish-tissue homogenate was then taken and centrifuged at 2500 rpm for 3 to 5 minutes. After centrifugation, the supernatant was taken for bacteriological analysis.

Isolation and Identification of bacteria from samples

The isolation and identification of bacteria from the collected samples was done on the basis of routine cultural, morphological and biochemical tests, such as colony characteristics which were interpreted as per described by Merchant and Packer, (1967), morphology and staining which was accomplished by Gram's staining methods (Merchant and Packer, 1967; Cheesbrough, 2006), motility using Motility Indole Urea (MIU) medium (Cheesbrough, 1985), hemolytic activity as described by Gerhardt *et al*. (1994), and a series of biochemical tests, *viz*., sugar fermentation test (Cheesbrough, 2006), catalase test, Indole test, methyl red (MR) test, and Voges-Proskauer (VP) test (Cheesbrough, 1985).

Morphological identification by Gram's staining

Gram's staining of the pure culture was performed according to method described by Cheesbrough (2006). Briefly a single colony was picked up with a bacteriological loop smeared on a glass slide and fixed by gentle heating. Crystal violet was then applied onto smear to stain for two minutes and then washed with running tap water. Few drops of Gram's iodine were then added for few seconds. After washing with water, Safranin was added as counter stain and allowed to stain for 2 minutes. The slides were then washed with water, blotted and dried in air and then examined under light microscope (400X) using immersion oil.

Motility test

The motility test was carried out to differentiate motile bacteria from non-motile one (Cheesbrough, 1985). This test was performed in Motility Indole Urea (MIU) medium (Hi-media, India), where a sterile wire was used to inoculate 5 ml of sterile MIU medium taken earlier in a test tube with a smooth pure colony of the test organism. During inoculation into MIU medium, it was made with a sterile wire and stoppered the tube followed by incubation at 37°C for overnight. Motile bacteria were identified by spreading turbidity from the stab line or turbidity throughout the medium (compared with an un-inoculated tube). A negative control was used in this test.

Maintenance of stock culture of isolated bacteria

Stock cultures of the isolated bacteria were prepared following the method described by Siddique *et al*. (2017). Firstly, bacterial single colony was taken by the sterile inoculating loop and was inoculated into sterile nutrient broth and incubated at 37°C temperature for overnight and examined for the turbidity. One millilitre of broth culture was taken in a sterile eppendorf tube with a micropipette and centrifuged at 10,000 rpm for 5 minutes and the supernatant was discarded. For washing 300 µl PBS was added to the eppendorf tube, mixed well and further centrifuged at 10,000 rpm for 5 minutes and the supernatant was discarded. After that 500 µl of PBS was added, mixed well and transferred to cryogenic vial (autoclaved). Then 200 µl of 70% glycerol (autoclaved) was added and mixed well. Finally each of the vials was labelled properly and stored at -20°C.

RESULTS AND DISCUSSION

Cultural characterization of the isolated bacteria

Cultural characterization was done by streaking of enriched inoculum (in nutrient broth) on primary culture medium, such as nutrient agar in this study, followed by selection of single colony and then again streaking on selective and differential culture. Repeated subcultiring on selective media were done to get pure culture. The cultural characteristics of the isolated bacteria from dried fishes are represented in the Table 1. After incubation at 37^0C for overnight, the colony morphology (size, shape, elevation, surface, consistency, texture, color, even odor, etc.) of the isolated bacteria were compared and interpreted with the findings of previous published literatures (Merchant and Packer, 1967; Gerhardt *et al*., 1994; Cowan, 1985). The findings of the cultural characterization revealed *Escherichia coli*, *Bacillus* spp., *Staphylococcus* spp., *Salmonella* spp., and different unidentified bacteria in the present study.

Table 1. Cultural characteristics of the isolated bacteria on different culture media

Suspected bacteria	Agar media used	Colony characteristics
Escherichia coli	NA medium	large, thick, greyish white, moist, smooth, opaque
	EMB agar medium	Large blackish blue with green metallic sheen
	MA medium	dry, dark pink in color and are surrounded with dark pink area
Bacillus spp.	NA medium	Circular, opaque, rough with jagged edges, some colonies were large grey white and granular with less wavy edge
	BA medium	Grey-white, opaque colonies with beta hemolysis
Staphylococcus spp.	NA medium	Transparent, whitish, yellowish, orange color and smooth colonies
	BA medium	No hemolysis, but pigmented colonies
Salmonella spp.	NA medium	opaque and smooth colonies with 2-4 mm in diameter
	S-S medium	Translucent, smooth, round with black center
	MA medium	Colorless, translucent, smooth, and raised colonies
	BGA medium	whitish colonies with pale red color

Legends: SN: Serial number; NA: Nutrient agar medium; EMB: Eosin methylene blue agar medium; MA: MacConkey agar medium; BA: Blood agar medium; S-S: Salmonella-Shigella agar medium; BGA: Brilliant green agar medium

Morphological characterization of the isolated bacteria

Morphological characterization was accomplished by Gram's staining technique and the results for different bacterial isolates are represented in the Table 2. The results of Gram's staining of *E. coli* in this study are similar with the findings of Thomas *et al*. (2005); similarly, the results for *Staphylococcus* spp. are same as reported by Brooks *et al*. (2002) and Sarker (2009), the results for *Salmonella* spp. are same as of Cheesbrough (1985) and Sannat *et al*. (2017), and results for *Bacillus* spp. are similar with the reports of Merchant and Packer (1967) and Cheesbrough (1985).

Table 2. Morphological characterization of bacterial isolates based on Gram's staining and motility test

Bacterial isolates	Staining properties	Shape	Arrangement	Category	Motility
Escherichia coli	Pink color	Short plump rods	Single, paired or in short chain	Gram-negative	Motile
Bacillus spp.	Violet color	Large bacilli	Arranged in chain	Gram-positive	Non-motile
Staphylococcus spp.	Violet color	Cocci	Tetrad or grapes like cluster	Gram-positive	Non-motile
Salmonella spp.	Pink color	Bacilli	Single, irregular	Gram-negative	Motile

Legend: SN: Serial number

Results of biochemical tests of the isolated bacteria

In biochemical test, *Staphylococcus* spp. produce only acid, no gas was observed in Durham's tube, MR, VP, and Catalase test positive which were identical with the findings of Brooks *et al*. (2002) and Merchant and Packer (1967). *Salmonella* spp. were negative in Indole and VP test and positive to MR test which were identical with the findings of Buxton and Fraser (1977) and Sujatha *et al*. (2003). The biochemical tests for *E. coli* showed that the isolates were able to ferment sugar and produced both acid and gas, MR, Indole test positive and VP test negative which were identical with the findings of Cheesbrough (2006) and Perez *et al*. (2000). *Bacillus* spp. were found to be positive to Catalase and VP tests, however, negative to Indole and MR tests, and ferment basic five sugars with the production of only acid, which are almost similar with the findings of Merchant and Packer (1967) and Cheesbrough (1985).

Table 3. The results of biochemical tests of the isolates bacteria from dried fish

Name of the organism	Basic Sugar fermentation test					Catalase test	Indole test	MR test	VP test
	DX	ML	L	S	MN				
Escherichia coli	AG	AG	AG	AG	AG	+	+	+	-
Bacillus spp.	A	A	A	A	A	+	-	-	+
Staphylococcus spp.	A	A	A	A	A	+	-	+	+
Salmonella spp.	A	A	A	A	A	+	-	+	-

Legends: DX: Dextrose; ML: Maltose; L: Lactose; S: Sucrose; MN: Mannitol; AG: Acid and gas; A: Acid; MR: Methyl-Red; VP: Voges-Proskauer; +: positive; -: negative.

Percent occurrence of isolated bacteria from dried fish samples

Among different bacterial genera, isolated from fried fish in this study, the highest occurrence was in case of *Staphylococcus* spp. (80%), followed by *Bacillus* spp. (56%), *Salmonella* spp. (48%), *E. coli* (44%) and unidentified bacteria (24%). Sultana *et al*. (2010) also reported the similar percentage of occurrence for *Staphylococcus* spp., *Bacillus* spp., and *Salmonella* spp., however, no report on *E. coli* in that study. The highest occurrence of *Staphylococcus* spp. in dried fishes is due to its lowest minimum a_w among the identified bacteria in this study (Tapia *et al*., 2020). The presence of *E. coli*, *Salmonella* spp., and *Staphylococcus* spp. in dried fish samples were also reported previously (Hasan *et al*., 2016; Hussain *et al*., 2018; Nur *et al*., 2020). Microbial growth in dried fishes could be a consequence of intentional-improper drying to get more profit as complete drying cause weight loss. However, dried fishes become contaminated especially from harvesting to marketing level, due to improper packaging and poor hygiene and sanitary practices (Paul *et al*., 2018). Moreover, use of low-quality raw fish for drying is also considered as enhancing factor for microbial growth (Hasan *et al*., 2016). The presence of *Salmonella* spp. in this study clearly indicates lack of hygiene practices (Sultana *et al*., 2010; Nur *et al*., 2020). The presence of *Bacillus* spp. in dried fishes is an indication of environmental contamination, as in Bangladesh, fish drying is practiced by keeping fish on sand of sea beaches, or keeping on elevated bamboo rack which might act as the sources of *Bacillus* spp. in dried fish (Reza *et al*., 2009). Moreover, sun-drying can reduce microbial load, but not effective to destroy bacterial spores (Paul *et al*., 2018). Improper packaging and storage facilities can be considered as one of the influential factors for microbial contamination (Islam *et al*., 2020).

Table 4. Occurrence of different bacterial agents isolated from dried fishes

Bacterial isolates	**No. of sample examined**	**No. of bacterial isolates found**	**Percent (%) of occurrence**
Escherichia coli	25	11	44
Bacillus spp.	25	14	56
Staphylococcus spp.	25	20	80
Salmonella spp.	25	12	48
Unidentified bacteria	25	6	24

CONCLUSION

From the present study, it may be concluded that various pathogenic or spoilage potential bacteria were present in dried fishes, sold at different retail markets within Dhaka city corporation areas. Pathogenic and molecular characterization of the identified bacteria should be considered for future study. For the development of dried fish sector as sustainable and profitable industry in the context of Bangladesh, several effective interventions are essential, such as, use of good quality raw fishes for drying, awareness about hygiene and sanitary practices at each step from production to marketing level, and scientifically reliable and economic packaging of dried fishes. Moreover, there should be an effective upstream intervention, such as market monitoring system to investigate the organoleptic, chemical and microbial safety of foods.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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