Int Aquat Res (2021) 13:173–184
https://doi.org/10.22034/IAR.2021.1924974.1142

ORIGINAL RESEARCH

Quality assessment and molecular identification of bacteria across the black tiger shrimp (*Penaeus monodon*) supply chains in Southern Bangladesh

Md. Sazedul Hoque . Md. Abu Sayed Ripon . Md. Belal Hossain . Md. Arifur Rahman . Md. Mahmudul Hasan . Biplob Dey Mithun . Avirup Saha . Suprakash Chakma . Mst. Niloy Jaman . Akhinur Rahman . Muhammad Manjurul Karim

Received: 03 March 2021 / Accepted: 10 July 2021 / Published online: 16 August 2021

Abstract Black Tiger Shrimp (*Penaeus monodon*) is one of the leading export commodities in Bangladesh. The industry, however, is frequently beset with quality deterioration and microbial diseases owing to various reasons. Here, we analyzed the sensory and nutritional qualities of shrimp, the total bacterial count, and molecular identification of pathogenic bacteria in samples collected from different supply chain actors (SCA), including the *gher* (farms), depots, wholesalers, retailers, and the processing industry in Khulna, Bangladesh. While the Sensory Quality Indices (SQI) of shrimps collected from farms, depots, and processing plants were found 'excellent and highly acceptable', the rating for wholesale and retail markets were 'good and acceptable'. Nutritionally, higher protein (21.59%), lipid (1.47%), and ash (1.97%) contents were found in shrimps collected from farms than that of other SCAs. Heterotrophic bacterial count (cfu/g) differed in various SCAs: farms 3.75±0.31 ($\times 10^6$), depots 3.95±0.92 ($\times 10^6$), wholesale markets 5.03±0.35 ($\times 10^6$), retail markets 4.82±0.40 ($\times 10^6$), and the processing industry 3.78±0.25 ($\times 10^6$). The presence of *Ralstonia* spp and *Pseudomonas* spp, confirmed by 16S rDNA sequencing were found in different SCAs. Overall, the quality of shrimp appeared better in farms than that of other SCAs, indicating that a lack of proper hygiene, sanitation, and post-harvest handling practices could be the likely impediments for quality deterioration.

Keywords Shrimp . Supply chain . Sensory quality . 16S rDNA sequencing . Microbial quality . Nutritional quality

Introduction

Bangladesh possesses immense potential with fisheries resources that posit it 3rd and 5th in global inland fisheries and aquaculture production, respectively (DoF 2019). Fish and fisheries products contribute greatly

Md. Sazedul Hoque (✉) . Md. Abu Sayed Ripon . Md. Mahmudul Hasan . Biplob Dey Mithun . Suprakash Chakma . Mst. Niloy Jaman
Department of Fisheries Technology, Patuakhali Science and Technology University, Dumki, Patuakhali-8602, Bangladesh
e-mail: sazedul.fst@pstu.ac.bd

Md. Belal Hossain
Department of Food Microbiology, Patuakhali Science and Technology University, Dumki, Patuakhali-8602, Bangladesh

Md. Arifur Rahman
Department of Fisheries Biology and Genetics, Patuakhali Science and Technology University, Dumki, Patuakhali-8602, Bangladesh

Avirup Saha
Bangladesh Food Safety Authority, Dhaka, Bangladesh

Akhinur Rahman . Muhammad Manjurul Karim
Department of Microbiology, University of Dhaka, Dhaka, Bangladesh

to food security in Bangladesh. The fisheries sector provides employment, meets 60% of total demand for animal protein, and contributes to the economy with 1.5% of total export earnings, 3.6% of national GDP, and 25.3% of agricultural GDP (DoF 2019). Of the exportable items, shrimp (*Penaeus monodon*) contributes greatly to Bangladesh's exports of fisheries products. Total shrimp and prawn production in 2017-18 was 250,000 metric tons, of which 53% was from cultured production (DoF 2019). Due to consumer preferences and market value in the international food supply chain, shrimp culture has become popular in the brackish-water habitats, abundant in the coastal districts of Bangladesh: Khulna, Satkhira, Bagerhat, Chittagong, and Cox's Bazar (Adhikari et al. 2015) from where shrimps are supplied to domestic consumers and abroad through different supply chain actors (SCA). Bangladesh exported about 36,168 metric tons of shrimp in 2018 that earned her more than US$ 400M (DoF 2019).

Globally, consumers are concerned about the quality (sensory, nutritional, and microbial) of food and fishery products (Huss et al. 2003). Quality-processed foods with minimal nutritional loss and attractive sensory properties receive higher consumer demand. Freshness is the primary quality of shrimp, and this can be observed in the context of color, texture, odor, and general appearance (Hossain et al. 2015; Olafsdottir et al. 2004). Basically, shrimp, or any seafood is nutritionally rich in its composition of protein and fatty acids, hence are considered essential for consumers; therefore nutritional state of shrimp either in fresh or in storage conditions constitutes a significant quality indicator (Soundarapandian et al. 2013).

After harvesting, shrimp and other seafood tend to lose sensory and nutritional qualities, and microbial contamination, if contracted could be carried through different supply chain actors causing further damage (Ali et al. 2013). In Bangladesh, the shrimp and prawn supply chains are traditionally handled by many intermediaries who provide different services (e.g., collection, icing, freezing, auction, or transportation). Several studies identified microbial agents that harmed the quality in shrimp, for example, the spoilage coliform, *Vibrio* spp, *Salmonella* spp, *Streptococcus* spp, *Staphylococcus* spp, and fecal coliform (Saima et al. 2012), and *Aeromonas* spp, *Klebsiella* spp and *Pseudomonas* spp (Samia et al. 2014) in shrimps sampled from different retail markets of Dhaka city, Bangladesh. However, no study was conducted to address the quality of shrimp at each point of the supply chain earlier. Here, we addressed the assessment of sensory and nutritional quality, and the bacterial prevalence in shrimp collected from different actors of the supply chain.

Materials and methods

Sample collection and preparation

Shrimp samples were collected from five different supply chain actors: the shrimp *gher* (farm), a depot, a wholesale market, a retail market, and a processing plant in Khulna division, Bangladesh referred to as G, D, W, R, and P, taking the first letters of the SCAs, respectively. Samples were collected in February 2018, a dry season that had a relative humidity of 65% and a temperature of 25°C on average. Three units of each supply chain actor were randomly employed for sample collection. Ten to twelve shrimps were collected from each supply chain unit, which were pooled together, thereby making about 30-35 shrimp samples that weighed 1 kg altogether from each SCA intended to analyze their respective sensory, nutritional, and microbiological qualities.These were transported to the Seafood Processing, Quality & Safety Lab at Patuakhal Science and Technology University (PSTU) within 4 hours after being packaged chill in sterile polythene bags carried in an icebox. The shrimp samples were then labeled and stored in a freezer at -18°C.

Sensory quality analysis

The sensory quality of the shrimp samples was analyzed using the Quality Index Method (QIM) with some modification of Martinsdóttir et al. (2001). The QIM was used to assess the freshness of the shrimp according to characteristics such as general appearance, shell color and softness, flesh color and consistency, odor, and physical damage (Table 1A). The sensory test was conducted by a panel of 10 trained post-graduate students. The panelists used a numerical score sheet to evaluate the shrimp quality. The numerical score (1-5) of each sample was averaged to determine the quality index score (Nowsad et al. 2015; Azam et al. 2013). The quality grades were defined based on the total number of defect points (DP) and defect

Table 1(A) Defect characteristics for sensory quality analyses

Characteristics		Defects	DP	Observations				
				S1	S2	S3	S4	S5
General appearance	a.	Fresh, bright and shining	1					
	b.	Slight loss of shininess	2					
	c.	Dull and loss of appearance	3					
	d.	Completely loose body	5					
Shell color	a.	Freshly caught, natural color	1					
	b.	Slightly dim or faded color	2					
	c.	Dim or faded, brownish color	3					
	d.	Completely faded, brownish to reddish	5					
Flesh color	a.	Freshly caught, transparent, shinning	1					
	b.	Slightly discolored, off-white to brownish	3					
	c.	Brownish to reddish musculature	5					
Meat odor	a.	Freshly caught odor	1 2					
	b.	Faint sour odor	2					
	c.	Moderate sour odor	3					
	d.	Strong sour odor	5					
Black discoloration of the shell or meat	a.	No black discoloration on shell or meat	1					
	b.	Few black spots on shell or shell joints	2					
	c.	Black spots on meat surface but not penetrated	3					
	d.	Black spots penetrated the meat	5					
Meat consistency	a.	Firm, elastic and consistent	1					
	b.	Not mushy but becoming loose	2					
	c.	Slightly mushy and tending to be loose	3					
	d.	Mushy, limp or floppy muscles	5					
Physical damage	a.	No broken head/neck/shell/telson	1					
	b.	Broken shell or telson but head and neck intact, exposed meat firm and elastic, no sign of spoilage	2					
	c.	Broken head and neck	5					

S1, S2, S3, S4 and S5 refer to the shrimp sample from each actor in the supply chain.

S1, S2, S3, S4 and S5 refer to the shrimp sample from each actor in the supply chain.

Table 1(B) Quality grading of shrimp in terms of the defect points

Grade	Defect points	Grade characteristics
A	<2	Excellent, Highly acceptable
B	2-3	Good, Acceptable
C	>3-<4	Deteriorating, Not acceptable
D	4-5	Spoiled, Rejected

characteristics (Table 1B).

The sensory assessment was calculated by using the following formula:

$$\text{Average Defects points} = \frac{\text{Total Defect Points}}{\text{Number of Defect Characteristics}}$$

Nutritional quality analysis

The nutritional quality of the shrimp sample from each supply chain actor was examined using the standard method of the Association of Official Analytical Chemists (AOAC 2005) procedures as mentioned below.

Protein

Crude protein content was determined following the Kjeldahl method (Method No. 978.04) (AOAC 2005). Reagents digestion mixture (100g Na_2SO_4/K_2SO_4, 10g $CuSO_4$ and 1g selenium powder), $Na_2S_2O_3$ (8%), concentrated H_2SO_4, H_3BO_3 (2%), NaOH (40%), standard HCl (0.1N), mixed indicator (0.2g methyl red and 0.1g methyl blue in 100ml ethanol) were prepared.

For the determination of crude protein by Kjeldahl apparatus (Bloc Digest 12, JP Selecta, Spain), the chopped shrimp sample (approximately 1.0 g) was taken in a clean Kjeldahl flask and 4.0 g of digestion mixture was added along with 25 ml of 98% H_2SO_4 by swirling the flask. Then the Kjeldahl flasks were placed in an inclined position on the heating device of Kjeldahl apparatus and were heated at 70°C for about 1-1.5 hours. The end-point of digestion was indicated by the appearance of a clear or light blue solution. The content of the flask was cooled at room temperature and 100 ml of distilled water and 25 ml of $Na_2S_2O_3$ were continuously added in each flask and were mixed and cooled. A few glass beads were added to each flask to prevent bumping. Then 100 – 120 ml of 40% NaOH was added to each flask to make the solution alkaline. The flask was immediately connected to the distilling bulb on the condenser. A conical flask containing 50 ml of 2% H_3BO_3 with 2 drops of the mixed indicator was placed under the condenser against Kjeldahl flask to collect the distillate. After completion of distillation, about 100 ml distillates were titrated with standard HCl. The end-point was indicated by light pinkish color. Total nitrogen was calculated by using the following formula:

$$\text{Nitrogen}(\%) = \frac{\text{ml. Acid titrate} \times \text{ normality of acid titrated} \times \text{ mili equivalent of N}(0.014)}{\text{Weight of sample}} \times 100$$

$$\text{Crude Protein}(\%) = \text{Nitrogen}(\%) \times 6.25$$

Lipid

Crude lipid was determined by the Soxhlet (J-SH3, JISICO, Korea) extract method using acetone as the extraction agent (60–80°C) (Method No. 930.09) (AOAC 2005). Samples (2-3g) were taken in thimbles and were dipped into a soxhlet tube. After boiling for 15 minutes, extraction with acetone was done for 3 hours and collected in pre-weighed round bottom-joined glass-flax. The glass-flax was then placed in an oven at 60-80°C until acetone is completely evaporated. The flax with lipid was cooled in a desiccator and weighed again. The lipid content (in percent sample) was calculated using the following formula:

$$\text{Lipid content}(\%) = \frac{\text{Weight of lipid}}{\text{Weight of sample}} \times 100$$

Ash

Ash content was assayed by incinerating the samples in a muffle furnace (HM-9MP, Raypa, Spain) at 550°C (Method No. 930.05) (AOAC 2005). About 3-5 g prepared sample was taken in a pre-weighed porcelain crucible and was placed in a muffle furnace at 550°C for 6 hours. Then the crucibles were cooled

in a desiccator. The remaining materials were taken as ash, and were calculated for each sample as per the following calculation:

$$\text{Ash content}(\%) = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Moisture

Moisture content was determined gravimetrically by drying the samples in a hot air oven (HAS/50/TDIG/SS, Hot Air Oven, Genlab, UK) at 105°C for about 24 hours, or until a constant weight was obtained. The loss of moisture was calculated as percent moisture using the following formula:

$$\text{Moisture content}(\%) = \frac{\text{Weight of wet material} - \text{Weight of dry material}}{\text{Weight of wet material}} \times 100$$

Bacteriological study

The shrimp samples were thawed, washed, and peeled before the carapace and telson were removed. Then the abdominal segments were chopped. The preparation of samples and the bacterial culture were conducted following the standard method of Schulze-Schweifing et al. (2014). Briefly, 10 g of mixed shrimp sample (comprising shrimp from each SCA) were homogenized (BK-HG160, Biobase, China) and mixed in 200 ml sterile phosphate-buffered saline (PBS, 10 mmol/L PO_4^{3-}, 137 mmol/L NaCl, and 2.70 mmol/L KCl, pH 7.4) producing a 5% (m/v) sample suspension. The suspension was mixed thoroughly using a vortex mixture (VM-1000, DLII, Taiwan) before it was centrifuged (DM0412, DLAB-SI, USA) at top speed for 1 min. The supernatant was collected and was maintained as a stock solution.

Total bacterial counts

The stock solution (5% suspension) was 10 fold serially-diluted up to 10^{-5} using 0.85% normal saline as diluent before 0.1 ml of each of them was spread plated onto nutrient agar media. After incubation at 37°C for 24 hours in the incubator (JSGI-10T, JSR, Korea), the colonies developed in the media were enumerated and results were recorded as cfu/g of shrimp sample using the following formula:

$$\text{cfu/g} = \frac{\text{No. of colonies} \times 10 \times \text{dilution factor} \times \text{volume of total stock solution}}{\text{weight of the fish sample}}$$

Bacteriological study on selective media

Aliquots of 0.1 ml from each of the sample sites, ‘G’, ‘D’, ‘W’, ‘R’ and ‘P’, after serial dilutions of their respective stock solutions were spread onto the MacConkey agar media. After overnight incubation at 37°C, a single colony appeared in MacConkey agar was taken for enrichment growth in Alkaline Peptone Water (APW) (Oxoid, UK) at 37°C for 3 hours. Taking single loops of bacterial culture from APW, it was inoculated to each selective media: EMB (Eosin methylene blue), XLD (Xylose Lysine Deoxycholate), TCBS (Thiosulfate-citrate-bile salts-sucrose agar) and Cetrimide agar (Oxoid, UK) (Fig. 1).

Molecular identification of isolates by 16S ribotyping

Total DNA was extracted from the pure culture of isolates using the boiling method as described earlier (Queipo-Ortuño et al. 2008). The concentrations of the extracted DNA were measured in a NanoDrop™ 800 spectrophotometer (Thermo Scientific, California, USA). For amplification of 16S rDNA from each

Homogenized samples were inoculated on MacConkey Agar and incubated for 24 h at 37°C

Sampling Site	G	D	W	R	P
Colony Characteristics On MacConkey Agar	+ (large, elevated, smooth, round and colorless colonies)	+ (large, elevated, smooth, round and colorless colonies)	+ (flat, round, small and green colored colonies with metallic sheen)	+ (flat, smooth, round, small and colorless colonies)	+ (flat, round, small and green colored colonies with metallic sheen)

Single colony was taken from each sample and inoculated in APW, incubated for 3 h at 37°C

Colony ID	g	d	w	r	p

One loop of Bacterial culture from APW was further inoculated in EMB, XLD, TCBS, Cetrimide agar and incubated for 24 h at 37°C

Growth on EMB agar	-	-	+ (flat, round, small and green colored colonies with metallic sheen)	+ (flat, rough, moderate and colorless colonies)	+ (flat, round, small and green colored colonies with metallic sheen)
Growth on XLD agar	-	-	-	-	-
Growth on TCBS Agar	-	-	-	-	-
Growth on Cetrimide Agar	-	-	-	+ (flat, rough, small and light green colored colonies)	-
Presumptive identification	Identification has to be confirmed by 16S ribotyping	Identification has to be confirmed by 16S ribotyping	*E. coli*	*Pseudomonas* spp	*E. coli*

Fig. 1 Process and result of presumptive identification of bacteria in shrimp

of the bacterial isolates, polymerase chain reaction (PCR) was carried out using the extracted DNA as template, the universal primer pair: forward (5'-AGAGTTTGATCMTGGCTCAG-3') and reverse (5'-GGTTACCTTGTTACGACTT-3') (Stevens and Elsas, 2010) and the method described in Sultana et al. (2020). Briefly, PCR was carried out using thermal cycler (model Aeris™, 96 wells, Esco Micro Pte. Ltd., Singapore) with the following amplification conditions: 94°C for 5 min for initial DNA denaturation, 35 cycles at 94°C for 1.5 min (denaturation), 56°C for 1 min (annealing) and 72°C for 1.5 min (extension), and a final extension step at 72°C for 10 min. The amplified products were analyzed by gel electrophoresis in a 1.0 % agarose gel (Sigma, USA) with a 100 bp DNA ladder (Invitrogen, USA) run concurrently as a marker, and visualized by using a gel documentation system (Alphaimager, USA).

Following the manufacturer's instructions, 16S rDNA PCR products were purified using the FavorPrep™ GEL/PCR Purification Kit (Favorgen, Taiwan). The purified products were sent for automated DNA sequencing (1st Base, Serdang Perdana, Malaysia). Partial sequences obtained using forward and reverse primers were assembled to get desired partial sequence (1400 bp–1500 bp) via the DNA Baser Assembler (v 4.36.0). The resulting sequence of the isolate was aligned with sequences in the GenBank database of the National Center for Biotechnology Information (NCBI) using the basic local alignment search tool (BLAST) to identify phylogenetically related microorganisms. Reference sequences were selected through BLAST with a high percentage of query coverage and identity. A phylogenetic tree was constructed using the selected reference sequences, sample sequence, and outgroup sequence aligned in the

Table 2 Sensory quality of shrimp from different SCAs

Source of shrimp	Sensory Quality Characteristics/ Defect Characteristics							Defect Point (DP)*	Grade	Grade characteristics
	General Appearance	Color of Shell	Color of flesh	Odor of meat	Black discoloration of shell or meat	Consistency of meat	Physical Damage			
Farm (gher)	Fresh, bright and shining	Natural greenish color	Freshly caught, transparent, shining	Freshly caught odor	Few black spots on shell or shell joints	Firm, elastic and consistent	No broken head/neck/shell/telson	1.14± 0.45^{c}	A	Excellent, highly acceptable
Depot	Fresh, bright and shining	Natural color	Slightly discolored, off-white to brownish	Moderate sour odor	No black discoloration on shell or meat	Firm, elastic and consistent	No broken head/neck/shell/telson	1.57± 0.38^{bc}	A	Excellent, highly acceptable
Wholesale market	Slight loss of shininess	Slightly dim or fade color	Slightly discolored, off-white to brownish	Faint sour odor	No black discoloration on shell or meat	Slightly mushy and tending to be loose	No broken head/neck/shell/telson	2.0± 0.24^{ab}	B	Good, acceptable
Retail market	Dull and loss of appearance	Slightly dim or fade color	Slightly discolored, off-white to brownish	Moderate sour odor	Few black spots on shell or shell joints	Slightly mushy and tending to be loose	No broken head/neck/shell/telson	2.43± 0.32^{a}	B	Good, acceptable
Processing plant	Fresh, bright and shining	Natural greenish color	Freshly caught, transparent, shining	Freshly caught odor	No black discoloration on shell or meat	Slightly mushy and tending to be loose	No broken head/neck/shell/telson	1.29± 0.35^{c}	A	Excellent, highly acceptable

*Mean values of DP were calculated from the evaluations of ten panelists. The different letters in the column for DP (shown in superscripts) indicate the significant ($P < 0.05$) differences in the sensory quality of shrimp from different actors in the supply chain.

Table 3 Nutritional quality of shrimp from different SCAs

Nutritional content (%)	Sources of sample				
	Farm(gher)	Depot	Wholesale market	Retail market	Processing plant
Protein	21.59^{a}±0.48	19.20^{b}±0.21	18.73^{b}±0.46	16.33^{c}±0.28	16.76^{c}±0.32
Lipid	1.47^{a}±0.12	1.20^{b}±0.17	1.38^{ab}±0.17	0.94^{c}±0.04	0.87^{c}±0.05
Ash	1.97^{a}±0.12	1.33^{b}±0.17	1.22^{b}±0.28	1.14^{b}±0.24	0.66^{c}±0.27
Moisture	76.25^{c}±0.85	78.35^{b}±0.62	78.20^{b}±0.34	78.30^{b}±0.14	80.24^{a}±0.08

Values are averaged from three observations (n=3). The different letters (shown in superscripts) in each row indicates the significant ($p<0.05$) differences of the same nutrient composition of shrimp from different SCAs.

MEGA 6 software. The percentage of replicate trees in which the associated taxa clustered together (bootstrap test = 1000 replicates) is shown next to the branches. The model to find evolutionary distance was set into the Kimura 2-parameter method (Kimura 1980; Kim et al. 2014).

Statistical analysis

All analyses were performed through SPSS (Statistical Package for Social Science) (SPSS Inc., Chicago, Illinois, USA) software version 23.0. All data are expressed as mean ± SD (standard deviation), and the differences between means were analyzed by Least Significant Difference (LSD, ANOVA). To compare all possible group differences Games-Howell nonparametric post hoc analysis approach was performed. Deviation of total viable bacterial counts (cfu/g) from the ICMSF (1986) standard was verified with Student's *t*-tests. Values with a $p < 0.05$ were assessed significantly different.

Results and discussion

Sensory quality of shrimp

The quality of the shrimp was examined on a scale of 1 to 5 according to the sensory quality analysis, and thereafter graded from 'A' to 'D' based on their defect points (Table 1). Here, we report shrimps, collected from the farm (gher), depot, and processing plant were of grade 'A' quality indicating their 'excellent and highly acceptable' sensory state, while that of wholesale and retail markets were considered grade 'B', i.e. of 'good and acceptable' quality (Table 2). Likewise, Akuamoah et al. (2018) reported that the color, aroma, texture, and taste parameters of shrimp from local market fell in the ranges of 1.04–2.0, 1.0–2.16, 1.08–2.12, and 1.64–2.2, respectively. Balfour et al. (2014) also found that an average sensory score of raw shrimp fell within the 'excellent' quality. López et al. (2013) reported bright color, excellent odor, elastic firmness, and very good quality of shrimp collected from the landing center/ wholesale market of Mexico. The current study also found comparatively better quality shrimp in depot and processing plant, thanks to the freshness of the shrimp. Furthermore, the processing plant and depot (which is mostly under a contract with the processing plant) usually contain better quality shrimp management owing to maintenance of a cold chain and good post-harvest handling practices to ensure quality export; this may not be the case for the two other SCAs: wholesale and retail market. Overall, this study reports 'good' to 'excellent' quality of shrimp from the sensory viewpoint collected from different SCAs in Bangladesh (Table 2).

Nutritional quality of shrimp

The nutritional quality of the shrimps collected from farm (gher) revealed that it had higher amount of protein, lipid and ash content, and lower moisture content compared to that of other SCAs, such differences were found statistically significant ($P < 0.05$) (Table 3). However, amounts of protein and lipid contents measured in shrimp sourced from the depot, wholesale market, retail market, and processing plant were similar ($P > 0.05$). Ash content was the highest in shrimp from the farm (gher) and lowest from that of the

Table 4 Total bacterial count in shrimp from different SCAs

Source of shrimp sample	Total viable counts (cfu/g)	ICMSF* (1986)	*p-value t-test	p-value (LSD, ANOVA)
Farm (gher)	3.75±0.31 ($\times10^6$) ^{cde}		0.004	
Depot	3.95±0.92 ($\times10^6$) ^{ac}		0.031	
Wholesale market	5.03±0.35 ($\times10^6$) ^{a}	$1x10^6$	0.003	0.027
Retail market	4.82±0.40 ($\times10^6$) ^{abc}		0.004	
Processing plant	3.78±0.25 ($\times10^6$) ^{bcd}		0.003	

Values are averaged from three observations (n=3). The different letters in the column (shown in superscript) for viable counts indicate the significant ($p<0.05$) differences in bacterial content from different SCAs, performed through Games-Howell nonparametric post hoc analysis approach. *p-value ($p<0.05$) represent significant difference between total viable counts from each of SCAs and ICMSF value, verified with Student's t-tests. Differences between SCAs' means were analyzed by Least Significant Difference (LSD, ANOVA) and values with a $p<0.05$ were assessed significantly different.

processing plant ($P < 0.05$), while shrimp from the other three sources had similar ash content ($P > 0.05$). The highest (80.24±0.08%) and lowest (76.25±0.85%) moisture content were evident in shrimp collected from the processing plant and farm (gher), respectively and this difference was statistically significant ($P < 0.05$). The findings of López et al. (2013) also reported the comparable composition of nutritional factors consistent with this study.

The differences in nutrient composition could be due to differences in sources, farming, post-harvest handling, preservation, and transportation practices across the supply chains. Reddy and Reddy (2014) also reported differences in sex, season, reproductive behavior, food source availability, period of capture, and hydrologic level that contributed to variations in the proximate composition.

Here we report that the higher nutritional content (protein, lipid, and ash) in shrimp from the farm is in line with corresponding better sensory characteristics. On the other hand, the shrimp from the processing plant was evidenced with the lowest nutritional content (protein, lipids, and ash; Table 3) despite the presence of higher sensory quality (Table 2) and lower bacterial load (Table 4). The reduced nutritional content could be favored by higher moisture content, which is due to the absorbance of water during the cold chain process (melting of ice used for storage and/or being soaked in iced water prior to processing). Furthermore, the melting water from ice carries a considerable fraction of soluble protein, salts, flavoring compounds and nutritional substances that could result in lowering nutritional content (Nowsad 2007). Ruth et al. (2014) reported that protein and lipid content varied due to presence of relative moisture fractions in fishes. Eventually, a study by Nowsad (2014) identified factors for producing satisfactory sensory quality of shrimp from processing plant, which are application of adequate icing, reduced handling, shorter supply chain and improved sanitary condition of labors. Comparatively, shrimp samples from the retail market had lower nutritional values with a lower grade of sensory quality. Overall, this study found the shrimp from the farm (gher) was the most nutritionally acceptable out of all supply chain actors and had premium food values.

Total bacterial counts in shrimp

The total viable bacterial counts approximately ranged from 3.75-5.03 million cells per gram of shrimp collected from different SCAs (Table 4). While the larger counts were recorded for the wholesale and retail markets, the opposite was evidenced for the farm (gher) and processing plant, and this difference was statistically significant ($p<0.05$). Comparable microbial loads in retail shrimp markets were reported in several studies (Rahman et al. 2012; Yousuf et al. 2008). The higher bacterial loads (Table 4) as found in shrimp samples from the wholesale market was demonstrated with deteriorated sensory (Table 2) and nutritional (Table 3) properties of the respective sample indicating the likely reasons could be the poor personnel hygiene and sanitation, and poor post-harvest handling (Pinu et al. 2007), as it passed more supply chain actors. On the contrary, shrimp from the farm was found to be of better sensory quality (Table 2) and had lower bacterial counts (Table 4). As far as the bacterial limit in raw crustacean set by the International Commission of Microbiological Standards for Food (ICMSF 1986) is concerned ($1x10^6$ cfu/g), the bacterial abundance in our samples regardless of the SCAs were found significantly higher (p

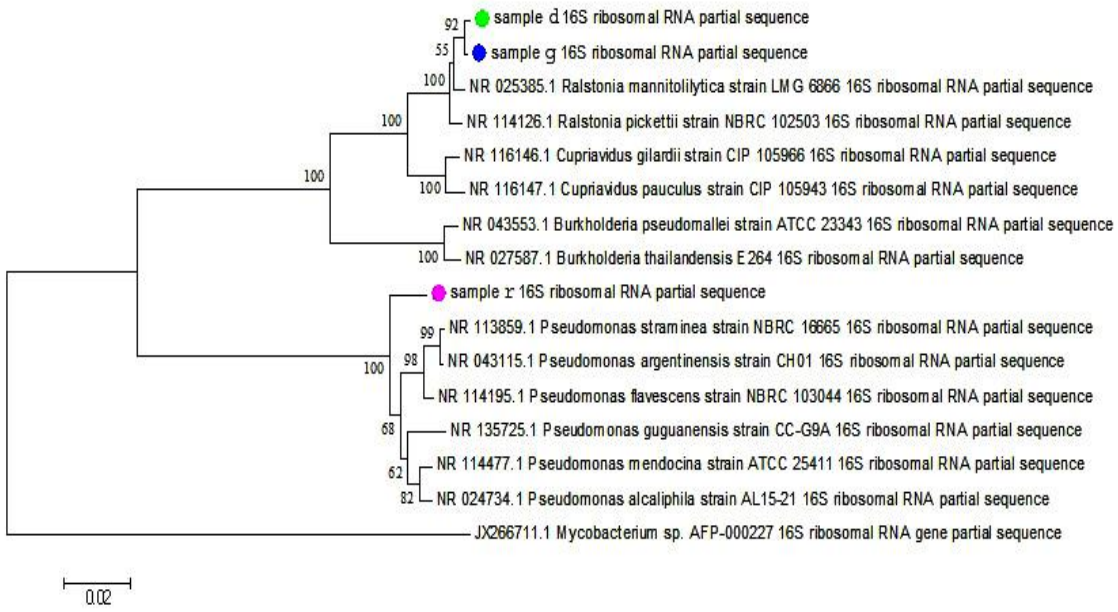

Fig. 2 Phylogenetic tree constructed from partial 16S rDNA sequences of the isolated strains

< 0.05) (Table 4). This further reiterates the necessity of maintaining good aquaculture practices (GAP) and improving post-harvest handling to bring down microbial loads within the standard limit.

Presumptive identification of bacterial isolates

All the samples showed growth on MacConkey agar where they produced large, elevated, smooth, round, and colorless colonies (lactose non-fermenter) from samples 'G' & 'D'; flat, smooth, round, small and colorless colonies (lactose non-fermenter) from sample 'R', and elevated, smooth, round, large and pink colonies (lactose fermenter) from samples 'W' and 'P' generally (Fig. 1). Next, single representative colonies from MacConkey agar produced from each sample type, IDed as 'g', 'd', 'w', 'r' and 'p' to represent from gher, depot, wholesale market, retail market, and processing plant respectively were inoculated onto EMB, TCBS, XLD and Cetrimide agar after being enriched in alkaline peptone water, and the selective growth was recorded (Fig. 1). While 'g' and 'd' showed no further growth on any selective media inoculated thereafter, 'w' and 'p' produced growth on EMB agar only, and 'r' produced growth in cetrimide agar in addition to EMB. Based on the microbial growth on selective culture media, 'w' and 'p' were presumptively identified as *Escherichia coli*, while 'r' can be identified as *Pseudomonas* spp. However, all these colonies need confirmed identification, hence were subjected to 16S ribotyping.

Phylogenetic analysis of 16S rDNA gene sequencing

Five presumptively identified isolates: 'g', 'd', 'w', 'r' and 'p' were subjected for sequencing of their 16S rDNAs, once amplified from their respective chromosomal DNAs. After sequencing, forward and reverse sequences were assembled using a DNA Baser Assembler (v 4.36.0). A phylogenetic tree was constructed from the partial sequences obtained based on the NCBI-BLAST result using MEGA 6 (Tamura et al. 2013) neighbor-joining tree (Fig. 2). A kimura-2 parameter was taken as a parameter to compute the evolutionary distance. The *Mycobacterium* spp AFP- 000227 strain was used to serve as an out-group for this analysis.

The isolates 'g' and 'd' aligned closely with *Ralstonia mannitolilytica.* This identification is well corroborated with their growth appeared in the MacConkey selective media (Fig. 1), supported by studies of Osterhout and Valentine (1998) and Pfeiffer and Jendrossek (2011). The isolate 'r' (retail market) matched closely with *Pseudomonas* spp, yielding an identity of 91-92%, and this is in line with its presumptive identification (Fig. 1). The smaller query coverage may be a reason why it produced a different branch.

However, the phylogenetic tree is self-contained enough to indicate its relatedness with *Pseudomonas* spp. Isolates 'w' (wholesale market) and 'p' (processing plant) failed to match with any bacterial 16S rDNA NCBI-BLAST partial sequences due to their poor quality of sequences. However, based on their presumptive identification as depicted in Fig. 1, these isolates can be identified as *E. coli*.

The presence of *Ralstonia* spp and *Pseudomonas* spp is a growing concern as emerging pathogens in shrimp aquaculture. In addition to *Escherichia coli*, *Klebsiella* spp., *Vibrio* spp., *Aeromonas* spp. and *Listeria* spp, the presence of *Pseudomonas* spp in a shrimp processing plant in Bangladesh (Rahman et al. 2012) and marine shrimp of India (Lakshmi et al. 2013) were reported as causes of concern. Although reported less frequently, the presence of *Ralstonia* spp was evident in diseased *Oreochromis niloticus* (Nile tilapia) and *Clarias gariepinus* (African catfish) in Uganda (Wamala et al. 2018), placing it a potential pathogenic agent for fishes. It may be mentioned that the presence of *Ralstonia* spp in shrimp and/or prawn is yet to be reported (Al Shabeeb et al. 2016), here, we produce evidence the first of its kind, for its presence in shrimp. Since *Ralstonia* spp is an opportunistic pathogen with the potential to cause human infection (Ryan and Adley 2014), it becomes a concern for shrimp aquaculture; appropriate measures are therefore needed to implement for its control and management to avoid large losses in the shrimp industry.

Conclusion

The sensory, nutritional, and microbial quality aspects of commercially significant shrimp in the Khulna region of Bangladesh varied depending on the stages in the supply chain from which the shrimp was sourced. Shrimp from the farm (gher) and the processing plant were found excellent in terms of nutritional composition, while those collected from the depot, wholesale market, and retail market were rated significantly below compared to that of gher. The higher count of total bacteria accompanied with the lower sensory and nutritional quality of shrimp from the retail market indicated poor post-harvest handling, processing, transportation, and sanitation practices along the supply chains. The presence of *Pseudomonas* spp, *E. coli,* and *Ralstonia* spp in the SCAs further demands the necessity of following good aquaculture practices throughout the shrimp supply chains.

Conflict of interest The authors declare no conflict of interest.

Acknowledgments The authors would like to acknowledge University Grant Commission Bangladesh (UGC) for the financial support provided for this research (Reference: UGC/Sci. & Tech./Agri.-Life-30/2016/2372).

Authors Contribution MSH: Design and conceptualization of the study; finalization of the draft; MASR: Collection and laboratory analysis of the sample and drafting the manuscript; MBH & MAR: Statistical analysis, and revision of the draft; MMH and BDM, Wet lab analysis; SC and MNJ: Data acquisition and interpretation; AS and AR: Dry lab analysis; and MMK: Coordination of the study and finalization of the manuscript.

References

Adhikari H, Ali MY, Shahiduzzaman M, Shams FI, Sarower MG (2015) Biochemical and PCR assay for detection of pathogenic bacteria at shrimp and shrimp farms in Bangladesh. Fish Aquac J 6:121

Akuamoah F, Odamtten GT, Kortei NK (2018) Sensory evaluation of gamma irradiated shrimp (*Penaeus notalis*) from three different water sources in Ghana. Int J Food Sci Nutr Diet 7:383-386

Ali MY, Mahmud Z, Rashed MA, Khanom M, Sarower MG (2013) Post-harvest quality loss of shrimp (*Penaeus monodon*) in the value chain of south western region (Satkhira) in Bangladesh. Intl J Sci Knwl 3:36-44

Al Shabeeb SS, Ibrahim MM, Al Ramadhan GH (2016) A comparative microbial quality assessment among fishes, prawns and cuttlefishes collected from Dammam fish market. Int J Curr Microbiol Appl Sci 5(9):405-418

AOAC - Association of Official Analytical Chemicals (2005) Official method of analysis, 12th edition, Association of Official Analytical Chemist, Washington DC, pp: 832

Azam AKMS, Mansur MA, Asadujjaman M, Rahman M, Sarwer MG (2013) Quality and safety aspects of fresh and frozen prawn (*Macrobrachium rosenbergii*), Bangladesh. Am J Food Technol 1(4):77-81

Balfour ST, Badrie N, Chang Yen I, Chatergoon L (2014) Microbiological, physical and sensory quality of marine shrimp (*Peneaus* spp.) sold by vendors in Trinidad, West Indies. Int Food Res J 21:1279-1288

DoF - Department of Fisheries (2019) National fisheries week compendium (In Bengali). Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh. pp: 15-25

Hossain MS, Hasan MM, Sarwer MG, Bhowmik S (2015) Comparative analysis of microbiological status between raw and ready-to-eat product of black tiger shrimp (*Penaeus monodon*). Int J Biosci 6:43-49

Huss HH, Ababouch L, Gram L (2003) Assessment and management of seafood safety and quality. pp: 239. Food and Agriculture Organization of the United Nations. Rome. FAO, Fisheries Technical Paper 444

Kim M, Oh HS, Park SC, Chun J (2014) Towards a taxonomic coherence between average nucleotide identity and 16S rDNA gene sequence similarity for species demarcation of prokaryotes. -Int J Syst Evol Microbiol, 64(2):346-351

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16(2):111–120

Lakshmi B, Viswanath B, Sai Gopal DVR (2013) Probiotics as antiviral agents in shrimp aquaculture. J Pathog, Article ID 424123, page 13

López DP, Palafox JTP, Quintero GB, Herrera MRT, Beltrán ER, Figueroa JLA (2013) A comparative study of physico-chemical, proximate composition and microbiological muscle properties, in two species shrimps of the pacific tropical coast. J Agric Sci Appl 2:151-154

Martinsdóttir E, Sveinsdottir K, Luten J, Schelvis-Smit R, Hyldig G (2001) Sensory evaluation of fish freshness. Reference manual for the fish sector. Ijmuiden, The Netherlands: QIM -Eurofish. p 49

Nowsad AKM A (2007) Participatory training of trainers: a new approach applied in fish processing. Published by Bangladesh Fisheries Research Forum. Chapter 9b, Chilling and icing of fish, pp 93-112

Nowsad AKM A (2014) Post-harvest fishery losses and mitigation measures. Published by Department of Fisheries Technology, Bangladesh Agricultural University. Chapter 08, Post-harvest loss in fish, pp 231-240

Nowsad AKM A, Hossain MM, Hassan MN, Sayem SM, Polanco JF (2015) Assessment of postharvest loss of wet fish: A novel approach based on sensory indicator assessment. SAARC J Agri 13(1):75-89

NCBI, http://www.ncbi.nlm.nih.gov/GenBank.

Olafsdottir G, Nesvadba P, Di Natale C, Careche M, Oehlenschläger J, Tryggvadóttir SV, Schubring R, Kroeger M, Heia K, Esaissen M, Macagnano A, Jørgensen BM (2004) Multi-sensor for fish quality determination. Trends Food Sci Technol 15:86-93

Osterhout GJ, Valentine JL, DickJD (1998) Phenotypic and genotypic characterization of clinical strains of CDC group IVc-2. J Clin Microbiol, 36(9):2618-2622

Pfeiffer D, Jendrossek D (2011) Interaction between poly (3-hydroxybutyrate) granule-associated proteins as revealed by two-hybrid analysis and identification of a new phasin in Ralstonia eutropha H16. Microbiol, 157(10):2795-2807

Pinu FR, Yeasmin S, Bari ML, Rahman MM (2007) Microbiological conditions of frozen shrimp in different food market of Dhaka City. Food Sci Technol Res 13:362-365

Queipo-Ortuño MI, Colmenero JDD, Macias M, Bravo MJ, Morata P (2008) Preparation of bacterial DNA template by boiling and effect of immunoglobulin G as an inhibitor in real-time PCR for serum samples from patients with brucellosis. Clin Vaccine Immunol: CVI, 15(2):293

Rahman MM, Rahman F, Afroze F, Yesmin F, Fatema KK, Das KK, Noor R (2012) Prevalence of pathogenic bacteria in shrimp samples collected from hatchery, local markets and the shrimp processing plant. Bangladesh J Microbiol 29(1):7-10.

Ruth SMV, Brouwer E, Koot A, Wijtten M (2014) Seafood and water management. Foods 3:622-631

Ryan MP, Adley CC (2014) *Ralstonia* spp.: emerging global opportunistic pathogens. Eur J Clin Microbiol Infect Dis 33(3):291-304

Reddy BS, Reddy KVS (2014) Proximate composition of the fresh water prawn *Macrobrachium rosenbergii* in cultured and frozen stage from Nellore Coast, India. Int Food Res J 21:1707-1710

Saima SN, Ghulam MM, Ahsan D, Khan MMR, Khan MAR (2012) Bacterial abundance in Indian white shrimp, *Penaeus indicus* collected from two different market conditions of Dhaka city, Dhaka University. J Biol Sci 21:29-38

Samia S, Galib HT, Tanvir AS, Basudeb CS, Walliullah M, Tasnia A, Sakil MM, Afsana FN, Sadia KP, Kamal KD, Mrityunjoy A, Nusrat JU, Tasmina R, Rashed N (2014) Microbiological quality analysis of shrimps collected from local market around Dhaka city. Int Food Res J 21:33-38

Schulze-Schweifing K, Banerjee A, Wade WG (2014) Comparison of bacterial culture and 16S rRNA community profiling by clonal analysis and pyrosequencing for the characterization of the dentine caries-associated microbiome. Front Cell Infect Microbiol 4:164

Soundarapandian P, Ravichandran S, Varadharajan D (2013) Biochemical Composition of Edible Crab, Podophthalmus Vigil (Fabricius). J Marine Sci Res Dev 3:119

Stevens P, van Elsas JD (2010) Genetic and phenotypic diversity of *Ralstonia solanacearum* biovar 2 strains obtained from Dutch waterways. Antonie van Leeuwenhoek, 97(2):171–188

Sultana S, Paul SC, Parveen S, Alam S, Rahman N, Jannat B, Hoque S, Rahman MT, Karim MM (2020) Isolation and identification of salt-tolerant plant growth-promoting rhizobacteria and its application for rice cultivation under salt stress. Can J Microbiol 66(2):144-160

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30(12):2725–2729

Wamala SP, Mugimba KK, Mutoloki S, Evensen Ø, Mdegela R, Byarugaba DK, Sørum H (2018) Occurrence and antibiotic susceptibility of fish bacteria isolated from *Oreochromis niloticus* (Nile tilapia) and *Clarias gariepinus* (African catfish) in Uganda. Fish Aquat Sci 21(1):6

Yousuf AHM, Ahmed MK, Yeasmin S, Ahsan N, Rahman MM, Islam MM (2008) Prevalence of microbial load in shrimp, *Penaeus monodon* and prawn, *Macrobrachium rosenbergii* from Bangladesh. World J Agric Sci 4:852-855

Publisher's Note

IAU remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.