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Original Research

Isolation and characterization of *Vibrio* sp from semi processed shrimp

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ABSTRACT:

This study was attempted to determine the Total Viable Count (TVC) and to characterize *Vibrio* sp in semi processed shrimp samples collected from the processing plant of Rupsha, Khulna. Randomly 20 shrimps were sampled each containing 4-5 shrimp from a processing plant at Rupsha, Khulna, aseptically removing the head and tail and transferred to the laboratory within icebox. After processing the sample by ten-fold serial dilution, spreading on nutrient agar plates were incubated for enumerating TVC. The shrimp muscle tissues were enriched after incubating within alkaline phosphate buffer solution and streaked onto TCBS agar and incubated for 24h at 37°C. Characteristic colonies (yellow colonies) were picked up and cultured onto nutrient agar for further characterization. Identification of *Vibrio* sp obtained from shrimp was performed by cultural, morphological and biochemical test. Antibiotic sensitivity profile of bacterial isolates was also performed against seven commonly used antibiotics by disc diffusion method. This study recorded that the Total Viable bacterial Count (TVC) varied from 7.00×10^5 cfu/g to 4.90×10^6 cfu/g and average TVC was found to be 2.13×10^6 cfu/g. Out of the 20 samples, five (25%) were found to be positive for *Vibrio* sp Antibiotic susceptibility test exposed that the isolates of *Vibrio* sp were resistant to ampicillin, intermediately resistant to erythromycin and sensitive to gentamicin, streptomycin, azithromycin, tetracycline and chloramphenicol. The findings of this study revealed that semi processed shrimp samples under this study were more or less contaminated with *Vibrio* sp which indicated the unhygienic condition of the shrimp culture and processing plant and the presence of antibiotic resistant bacteria in shrimp supposed to be a threat to food safety and might deteriorate the export quality.

Keywords:

TVC, *Vibrio* sp, Semi processed shrimp, Processing plant, Antibiotic sensitivity test.

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INTRODUCTION

Bangladesh as a river-irrigated country has got a history of fishing as well as aquaculture. The culture and consumption pattern of fish therefore has important ramifications for national income and food security (Ghose, 2014). In recent years, because of expanding universal interest, shrimp has turnout to be one of the most significant export items. Seafood holds the third rank position in all the export earnings whereas 85% of them come through shrimp (FAO, 2018). At the beginning, shrimp farming was practiced in the Southwestern part and Cox's Bazar district of Bangladesh. Bangladesh is supposed to be one of the most compatible places in the planets for prawn and shrimp cultivation, because of its huge resources of shallow water bodies which gives unprecedented opportunity for prawn and shrimp farming. Black Tiger (*Penaeus monodon*) and fresh water scampi (*Macrobrachium rosenbergii*) are the most widely exported shrimps among all the fishery commodities bringing the majority of foreign cash in this area.

Today the world is vouching the resurgence in the consumption of shrimp. During the last few years, scientists have reported cases of food-borne disease in humans caused by the utilization of contaminated shrimp, fresh raw shellfish and other sea foods. Poor hygienic condition in processing, preservation and storage may be the source of contaminating the shrimp (Huss *et al.,* 2000; Eze *et al*., 2011). Subsequently, different types of bacteria likes *Vibrio* sp, *Streptococci*, *Salmonella* sp coliform and *Staphylococcus* sp may contaminate the shrimp by spoiling and causing cholera and other foodborne disease outbreaks (Mobin *et al*., 2001). Periodically, *Vibrio* sp has been reported as the most critical reason of foodborne diseases, even it has been defined as a life threatening cause in some epidemic outbreaks (Colakoglu *et al.,* 2006; Raissy *et al.,* 2011). It becomes risk for human consumption when the presence of such pathogenic bacteria in shrimp exceeds the acceptable limits (Butt *et al*., 2004).

Vibrio is a genus of crustaceans commonly found in the aquatic habitat including shrimp culture ponds (Vijayan *et al*., 2006). Many *Vibrio* species are considered as pathogenic to human and responsible for causing foodborne disease (FDA, 1992). Species such as *V. cholerae, V. vulnificus, V. parahaemolyticus, V. alginolyticus, V. fluvialis, V. mimicus, V. metschnikovii, V. furnissii* and *V. damsela* are identified as human pathogens (Murray *et al.,* 1995). They cause severe infections in human beings like gastroenteritis, normally related with undercooked or raw seafood consumption, otitis, wound infections and septicemia (Murray *et al.,* 1995). *V. cholerae* and *V. parahaemolyticu*s are the main reasons for gastrointestinal illness in human while others cause non-intestinal illness.

Shrimp export market of Bangladesh is jeopardized for low nature of processed shrimp products which might be deteriorated due to hollow handling and responsible for food borne diseases (Nilla *et al.,* 2012). This study was conducted to evaluate the microbiological assessment of semi processed shrimp collected from fish processing plant at Rupsha, Khulna with special emphasis on the isolation of *Vibrio* sp in such shrimp samples. It is also attempted to study antimicrobial sensitivity patterns of the *Vibrio* species isolated from shrimps.

MATERIALS AND METHODS

Sampling

Total of 20 shrimp samples were collected from a processing plant at Rupsha, Khulna randomly from 20 different lots within a time frame of July, 2015 to August, 2015 aseptically using sterile hand gloves and polythene bag. Four to five shrimps from each sampling site were taken to perform microbial assessment and transferred to the laboratory within the icebox. The collected shrimp samples are considered as semi-

Table 1.Total viable count of semi processed shrimp samples collected from processing plant

S. No	Sample No	TVC (cfu/g)	Average TVC (cfu/g)	Permeable bacterial load in raw frozen shrimp
1	S-1	$8.80x10^5$		
2	S-2	$2.10x10^6$		
3	S-3	$9.60x10^5$		
4	S-4	$4.16x10^6$		
5	S-5	$4.90x10^6$		
6	S-6	$1.98x10^6$		
7	S-7	$1.70x10^6$		
8	S-8	$2.18x10^6$	$2.13x10^6$	$\leq 10^6$
9	S-9	$1.02x10^6$		(ISMSF, 1986)
10	S-10	$1.54x10^6$		
11	S-11	$1.90x10^6$		
12	S-12	$1.30x10^6$		
13	S-13	$3.96x10^6$		
14	S-14	$1.00x10^6$		
15	S-15	$2.22x10^6$		
16	S-16	$1.56x10^6$		
17	S-17	$2.40x10^6$		
18	S-18	$7.00x10^5$		
19	S-19	$3.76x10^6$		
20	S-20	$2.42x10^6$		

processed because the samples collected are not the final product of the shrimp processing plant. Shrimp samples were prepared after removal of the head and tail.

Bacterial enumeration

5 g of shrimp samples were mixed with 45 mL Phosphate Buffer Saline (PBS) and grinded by mortar and pestle. After that ten-fold serial dilution was done by using whirly mixture machine ranging from 10^{-1} to 10^{-5} according to the standard method (ISO, 1995). 50 µl from each ten-fold diluted sample was transferred and spread on the nutrient agar using a micropipette for the determination of Total Viable Count (TVC). Colony ranged from 30-300 was counted after overnight incubation and calculated according to ISO (1995). The result of TVC was recorded as colony forming unit per gram (cfu/g) of shrimp samples.

Isolation and identification

Suspension made of shrimp samples and Phosphate Buffer Saline (PBS) were incubated for enrichment of *Vibrio* sp at 37°C for 18-24 h. Isolation of *Vibrio* sp was performed by streaking a loop of suspension on selective media *viz.* Thiosulphate Citrate Bile salt Sucrose (TCBS) agar. Following incubation, plates were examined for colony morphology. Single well defined colony was further sub-cultured onto TCBS agar until pure colony was obtained. Gelatin agar (GA), Blood agar and MacConkey agar supplemented with 2% NaCl were used as the non-selective medium for purification of *Vibrio* sp (FDA, 1992).

For further identification, the predicted *Vibrio* sp isolates were subjected to morphological test by Gram's staining and biochemical tests including oxidase test, carbohydrate fermentation tests, catalase test, MR-VP test and indole test according to the procedures recommended by Bergey *et al.* (1974).

Salt tolerance test

The 0%, 1% and 6% NaCl salt containing nutrient broths were inoculated by very small amount of fresh culture. The inoculum was light enough so that visible turbidity of the salt containing broth was not hampered before inoculation. The broths containing different concentration of NaCl were incubated at 37°C for 18 to 24 h and the results were read after the incubation. The growth of bacteria was observed in the solutions that became turbid and those with no growth remained clear (Choopun *et al.,* 2002).

Antimicrobial sensitivity test

Antimicrobial drug susceptibility against seven commonly used antibiotics was performed by disc diffusion or Kirby–Bauer method (Bauer *et al.,* 1966). The zones of inhibition of bacterial growth were compared with the zone-size interpretative table (Table 3) provided by Clinical and Laboratory Standards Institute (CLSI, 2007).

Table 2. Biochemical properties of the isolated *Vibrio* sp

S. No	Biochemical test	Sample no				
		S-2	S-5	S-11	S-14	S-19
1	Catalase	+	+	+	+	+
2	Oxidase	+	+	+	+	+
3	Methyl red	+	+	+	+	+
4	Voges-Proskauer	-	-	-	-	-
5	Indole test	+	+	+	+	+
6	Glucose utilization	A	A	A	A	A
7	Dextrose utilization	A	A	A	A	A
8	Lactose utilization	-ve	-ve	-ve	-ve	-ve
9	Sucrose utilization	A	A	A	A	A
10	Mannitol utilization	A	A	A	A	A
11	Growth at 0% NaCl	+	+	+	ND	+
12	Growth at 1% NaCl	+	+	ND	-	+
13	Growth at 6% NaCl	-	-	-	-	-

Legends: + = Positive; - = Negative; A = Acid; -ve = Negative and ND = Not detected

RESULTS AND DISCUSSION

Almost all shrimp samples (n=20) exhibited higher microbial loads within a range of 7.00×10^5 cfu/g to 4.90×10^6 cfu/g (Table 1). In this study, the average Total Viable Count (TVC) was found to be 2.13×10^6 cfu/g. TVC of almost all samples were exceeded the safety limit of 10^6 cfu/g (ICMSF 1986). Yousuf *et al.* (2008) recorded TVC of $2.04x10^2$ to 4.5×10^5 cfu/mL for tiger shrimp (*Penaeus monodon*) from the District of Cox's Bazar and Satkhira and 1.08×10^2 to 1.2×10^5 cfu/mL for the giant water prawn (*Macrobrachium rosenbergii*) from Noakahli District in Bangladesh that was slightly lower than the current study. Nayem *et al.* (2011) and Samia *et al.* (2014) also reported TVC of 6.8×10^4 to 1.6×10^6 cfu/g and 1.5×10^4 cfu/g to $3.3x10^8$ cfu/g from giant fresh water prawn and shrimp of different local market in the Dhaka city respectively. The study revealed that the overall quality of the semi processed shrimp samples were not satisfactory which might have a great effect on the overall public health.

In the present study the colony characteristics of *Vibrio* sp observed on TCBS agar were yellow, shiny, round, smooth, glistening and slightly flattened (Figure 1) which is characteristically similar to those reported by Kumar *et al*. (2012) and Islam *et al*. (2013). Again the colony of *Vibrio* sp observed on non-selective media

Table 3. Antimicrobial sensitivity profile of isolated *Vibrio* sp

S. No	Sample no.	AMP (25µg)	GEN (10µg)	S (10µg)	AZM (30µg)	TE (30µg)	E (15µg)	C (30 µg)
1	S-2	R	S	S	S	S	I	S
2	S-5	R	S	S	S	S	I	S
3	S-11	R	S	I	S	S	S	S
4	S-14	R	S	S	S	S	I	S
5	S-19	R	S	I	S	S	I	S
6	Ref-R	≤13	≤12	≤11	≤11	≤11	≤13	≤12
7	Ref-I	14-17	13-14	12-14	12-14	12-14	14-22	13-18
8	Ref-S	≥18	≥15	≥15	≥15	≥15	≥23	≥19

Legends: Ref-R = Reference resistance; Ref-I = Reference intermediate; Ref-S = Reference sensitive; AMP = Ampicillin; GEN = Gentamycin; S = Streptomycin; AZM = Azithromycin; TE = Tetracycline; E = Erythromycin and C = Chloramphenicol

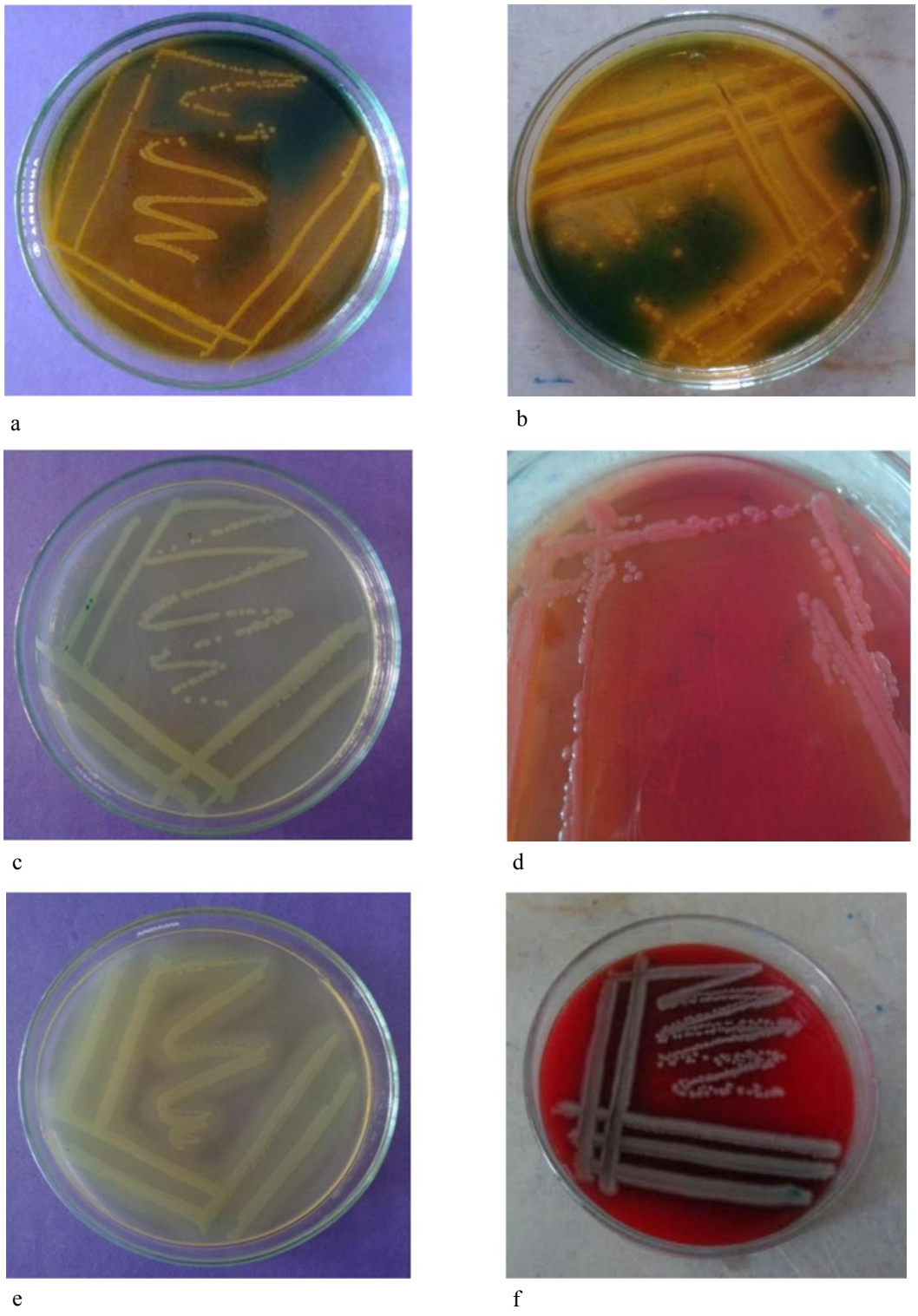

Figure 1. Cultural characteristics of presumptive *Vibrio* sp in different bacteriological media a, b: Yellow, shiny colonies, 2 to 4 mm in diameter on TCBS agar; c: Translucent, smooth colonies in Nutrient Agar (NA); d: Colorless to light pink colonies in Mac Conkey agar; e: Smooth, white colonies surrounded by opaque zone in gelatin agar; f: Colorless colonies with hemolysis in blood agar

such as gelatin agar, blood agar and MacConkey agar recorded in this study was agreed (Figure 1) with the findings of Islam *et al*. (2013). In Gram's staining, the morphology of the isolated *Vibrio* sp exhibited Gram

negative, small curved or rod shaped appearance under microscope which was supported by Faruque and Nair (2008) and Islam *et al*. (2013). All isolates were positive to oxidase test, indole test, catalase test, and MR test and negative to VP test (Table 2) which was found similar with the finding of Mahbub *et al*. (2011) and Nguyen *et al.* (2012). *Vibrio cholerae* isolates were able to ferment the glucose, inositol, mannitol, and sucrose by producing only acid (Nguyen *et al.,* 2012). In our study, *Vibrio* sp produced acid in dextrose, glucose, sucrose and mannitol but not in lactose which were found similar with the finding of Hossain *et al.* (2010). In salt tolerant test the isolated *Vibrio* sp grew in absence of NaCl, but their growth were stimulated by the addition of 1% NaCl where no growth was observed in 6% NaCl.

Out of the 20 samples, five (25%) were found to be positive for *Vibrio* sp. The above result was quite similar with the findings of Sathiyamurthy *et al.* (2013), reported 27.0% *Vibrio* sp in different environmental and seafood samples of Tamil Nadu, India. Adebayo-Tayo *et al*. (2011) isolated and identified *Vibrio cholerae, Vibrio parahaemolyticus, Vibrio fluvialis, Vibrio mimicus,* and *Vibrio vulnificus* from fresh the seafood where *Vibrio cholera* was most predominant 30.4%.

In this study, the isolates of *Vibrio* sp were tested against seven antimicrobial agents to observe whether they were sensitive or resistant to those agents. From the result it has been found that most of the isolates of *Vibrio* sp were resistant to ampicillin, intermediate resistant to erythromycin and sensitive to streptomycin, gentamicin, tetracycline, azithromycin and chloramphenicol (Table 3). More or less similar result was observed by Mahbub *et al*. (2011), who reported the highest resistance to ampicillin (100%) followed by amoxicillin (78%), nalidixic acid (40%), vancomycin (13.33%), neomycin (6.66%) and chloramphenicol (6.66%). The results of the present experiment proposed that commercially accessible fish may encourage the dispersal of the antibiotic resistant microorganisms. The principle reason of antibiotic resistance bacteria in shrimp might be the vigorous use of antibiotic in shrimp farming. Thus, effective medication must be ensured during fish borne disease outbreaks and the antibiotic susceptibility may be helpful for effective medication.

The present study was focused mainly on the isolation and characterization of *Vibrio* sp from semi processed shrimp samples by using conventional methods. Due to the limitation of resources, molecular study could not be done with the isolates that have shown positive results in conventional methods. So a long-term monitoring and screening program for the detection of pathogenic *Vibrio* sp in seafood industry in Bangladesh are required. In such program, application of molecular methods is strongly recommended to confirm the findings of conventional methods of identification and to differentiate pathogenic strains of *Vibrio* sp.

CONCLUSION

Based on the obtained results, this study may conclude that, there is a high risk of *Vibrio* sp contamination in seafood consumed by people in Bangladesh. To reduce the risk of getting infected by *Vibrio* species, it is necessary to avoid raw or insufficiently cooked seafood and marine products. Furthermore, we need to strictly control and monitor the storage temperature and establish hygienic handling practice of seafood.

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