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Identification, prevalence and antimicrobial susceptibility of major pathogenic bacteria in exportable shrimp (*Penaeus monodon*) of southern Bangladesh

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Abstract
A total of 30 samples of shrimp were collected from two local sea food processing plant of Rupsha and Mongla Bazar of Khulna district to investigate the microbiological quality. Microbial quality was assessed by total viable count (TVC), total *Vibrio* count (TViC), total Staphylococcal count (TSC) and total *E. coli* count (TEC) by inoculating into specific media and the values of all the samples were ranged from log 2.92 to 3.41cfu/gm, log 2.06 to 2.11 cfu/gm, log 2.21 to 2.49 cfu/gm and log 2.14 to 2.22 cfu/gm respectively. Out of 30 samples 10, 11 and 5 samples were found positive for *Vibrio* spp., *Staphylococcus* spp. and. *E. coli* respectively. Results of antibiotic susceptibility test against eight commercially available antibiotics showed all three bacteria were multidrug resistant. This study revealed that the shrimp samples of Mongla contain higher load of bacteria than the samples from Rupsha.

Keywords: shrimp, microbial quality, antibiotic sensitivity

1. Introduction
Bangladesh is blessed with natural gifts like aquatic resources. People of this country are largely dependent on fish culture and other fishery items those are now absolute part of their life style and culture. Aquaculture and aquatic resources are also ecologically, economically and aesthetically significant to this country. Presently there are 260 fish species of freshwater, 12 exotic fish species type, 475 types of marine fish species and 60 shrimp and prawn species available here [1]. Bangladesh has obtained 5.24% of her GDP, 63% animal protein supply and earned 4.76% foreign exchange from the fishery sector [1]. According to the geography and seasonal variations fishing activities in Bangladesh is strengthened and fish harvest attains peak in winter and late winter both from fresh water and marine water of the coastal crisscross channels. Among various fishes shrimp cultivation looms up in winter when large amount of fresh shrimp caught every day. So, shrimp processing reaches peak on winter and it continues until the beginning of the rainy season. According to Chandra [1], contribution of shrimp to the GDP of Bangladesh is 4.7% and among total export earning about 8% comes from shrimp. Therefore, considering the health safety and economical sustain of the consumer it is worth to maintain the microbial load and quality of the shrimp.
Shrimp is now commercially processed in Marine Food Processing industry. After removing head and tail, washing with chlorinated water shrimp is called Semi processed shrimp. Shrimps quality deteriorates at the very beginning of processing because of improper handling. After that further processing of same product cannot bring back its satisfactory freshness. Contamination in shrimp may be due to improper hygienic condition like inappropriate processing, preservation and storage condition [2]. As a consequence of this, processed shrimps may get contaminated with different types of bacteria such as *Vibrio* spp., *Salmonella* spp., coliform, fecal coliform, streptococci and *Staphylococcus* spp. those spoil shrimps and cause cholera and other food borne disease outbreaks.
Shrimp takes a large number of bacteria into their gut from water, sediment and food. *Bacillus* spp., *Salmonella* spp., *Shigella* spp., *Enterobacter* spp., *Escherichia* spp., *Micrococcus* spp., *Flavobacterium* spp., *Staphylococcus aureus, Pseudomonas* spp., *Rhizopus* spp., *Aspergillus*

spp., *Mucor Mucedo and Saccharomyces* spp. were isolated from shrimp [3]. *Vibrio sp.* has been isolated from aquatic environments in various part of the world. Many Staphylococcal strains vegetate harmlessly on skin and mucous membranes and as a foodborne pathogen may cause severe pneumonia and septicemia [4].

An important factor is virulent drug resistant gene of the spoiling micro flora that is concerned with shrimp culture and consumption which may pose serious health threat specially in treating diseases. Therefore the present study was designed to assess the microbial load and identification of the bacteria with their antimicrobial susceptibility status from shrimp processed at Shrimp processing plant at Khulna region.

2. Materials and Methods

Thirty samples were collected from two Shrimps processing plant (Rupsha and Mongla) of Khulna Region. After transporting to the laboratory in ice samples were processed and total viable count (TVC), total Staphylococcal count (TSC), total *E. coli* count (TEC) and total *Vibrio* count (TViC) were performed three times for each sample according to ISO [5]. The representative bacterial colonies were characterized morphologically using Gram's staining [6]. Catalase test, Coagulase test, Sugar fermentation test, Indole test, Methyl red test (MR) and Voges-Prouskauer (VP) test were conducted according to the method described by Cheesbrough [7]. All the isolates of *Staphylococcus* spp., *Vibrio* spp. and *E. coli* were tested for antimicrobial susceptibility test against eight commonly used antibiotics by disc diffusion or Kirby-Bauer method [8] and the results were compared with the standard [9].

The data on total viable count (TVC), total *Vibrio* count (TViC), total Staphylococcal count (TSC) and total *E. coli* count obtained from the bacteriological examination were analyzed in completely randomized design (CRD) using computer package subjected to analysis of variance using SPSS Software (Version 16, 2007).

3. Result and discussion

3.1 Microbial load

In the present study, TVC of bacteria in shrimp samples collected from two processing plant of Khulna ranged between log 2.92±0.22 cfu/gm in Rupsha and log 3.41 ± 0.23 cfu/gm in Mongla can be connected to a number of factors such as improper handling and processing, use of contaminated water, cross contamination from dirty processing, utensils like knife and trays. The total *Vibrio* count in all samples varied between log 2.06±0.09 cfu/gm and log 2.11± 0.06 cfu/gm in Rupsha and Mongla respectively. Total Staphylococcal count obtained from the study ranged between log 2.49 ±0.07 cfu/gm and log 2.21± 0.08 cfu/gm in Rupsha and Mongla respectively. Total *E. coli* obtained in this study ranged from log 2.22±0.10 cfu/gm to log 2.14±0.02 cfu/gm. The *E. coli* load was found in both shrimps sample from two areas of Khulna, which indicates the samples are not free from fecal contamination. The TVC and TViC were highest in Mongla and TSC and TEC were in Rupsha area. The TVC, TSC, TEC and TViC loads in all samples were found within acceptable range [10] and the results were presented in table 1. The findings of current study agreed with Ramesh *et al.* [11], recorded TBC ranged from log 2.25 to 2.5 cfu/gm and TCC was found in between log 2.33 to 2.71 cfu/gm in five commercially important shrimp samples in India. Hossain *et al.* [12] also reported TBC of log 5.27 cfu/gm and log 4.23 cfu/gm in raw black tiger shrimp and ready-to-eat product of shrimp respectively. But the results observed by Nayem *et al.* [13] was higher than the present study, recorded TVC, TCC, total fecal coliform and TViC ranged from log 4.83 to 6.21 cfu/gm, log 2.17 to 5.25 cfu/gm, log 0 to 2.69 cfu/gm and log 0 to 2.55 cfu/gm from giant fresh water prawn respectively. The presence of higher bacterial loads indicates the shrimp is contaminated with pathogenic microorganisms that deteriorate the quality of shrimp during processing, storage and distribution.

Table 1: Total bacterial load (TVC, TSC, TEC and TViC) of Shrimp samples collected from different area of Khulna Region

Sampling area	Sample no.	Mean log cfu±SD/gm			
		TVC	TViC	TSC	TEC
Rupsha	15	2.92±0.22	2.06±0.09	2.49±0.07	2.22±0.10
Mongla	15	3.41±0.23	2.11±0.06	2.21±0.08	2.14±0.02
P value		0.439	0.439	0.439	0.068
Permeable load (ICMSF, 1986)		$\leq 10^7$ cfu/gm	$\leq 10^3$ cfu/gm	$\leq 10^4$ cfu/gm	≤500 cfu/gm

Legends: SD= Standard deviation, TVC= Total viable count, TViC= Total *Vibrio* spp. count, TSC= Total *Salmonella* spp. count and TEC= Total *E. coli* count

2.2 Isolation and identification of bacteria

In the current study, three different genera of bacteria including 10 of *Vibrio* spp., 5 of *E. coli* and 11 of *Staphylococcus* spp. were isolated and identified. Isolation was performed on their selective agar media. Thiosulphate citrate bile salt sucrose (TCBS) agar was used to isolate the Vibrio spp. where it appears as yellow, round and smooth colony (fig 1) that is characteristically similar to findings of Islam *et al.* [14]. The colony characteristics of *E. coli* observed on eosin methylene blue (EMB) agar were smooth, circular, black color with metallic sheen (fig 2) and *Staphylococcus* spp. on mannitol salt (MS) agar showed yellow, round and convex colony respectively (fig 3). The cultural characteristics of *E. coli* and *Staphylococcus* spp. observed by Konuku *et al.* [15] and Sharada *et al.* [16] was similar with the study. Gram's staining and biochemical test were used to identify the bacteria. In Gram's staining, *Vibrio* spp. appeared as single curved shaped rod, *E. coli* showed pink color, small shaped gram negative and *Staphylococcus* spp. was cocci shape arranged in grapes like cluster. Islam *et al.* [14], Thomas *et al.* [17] and Brooks *et al.* [18] also recorded similar staining characteristics of *Vibrio* spp., *E. coli* and *Staphylococcus* spp. All the isolates of *Vibrio* spp. produced acid by fermenting dextrose, maltose, sucrose and mannitol except lactose. *Vibrio* spp. also gave positive reaction to methyl red (MR) and indole production tests and negative reaction to Voges–Proskauer (VP) test. *Staphylococcus* spp. was found catalase and coagulase positive whereas *E. coli* fermented dextrose, lactose, maltose, sucrose and mannitol with the production of gas and acid. The results of biochemical test agreed with the findings of previous researcher [15, 19-20].

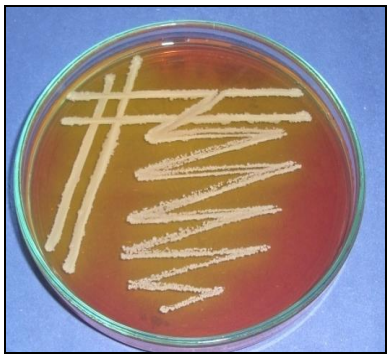

Fig 1: Small whitish or yellowish colonies of *Staphylococcus* spp. on MS agar

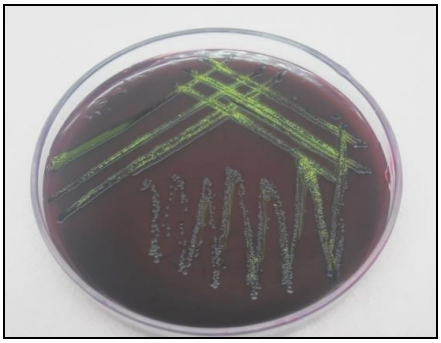

Fig 2: Smooth, circular, black or green color colonies with metallic sheen *E. coli* on EMB agar

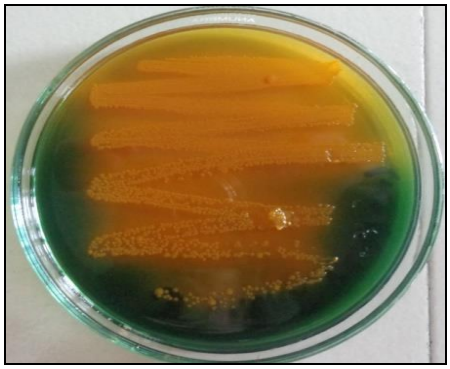

Fig 3: Yellow, shiny colonies of *Vibrio* spp. on TCBS agar

2.3 Antimicrobial susceptibility test

Antibiotic susceptibility test was performed against three bacteria using eight commercially available antibiotics. All *Vibrio* isolates were susceptible to gentamycin, azithromycin, ciprofloxacin, and tetracycline and resistant to amoxicillin (100%). Most of the isolates of *Staphylococcus* spp. were susceptible to azithromycin, tetracycline and gentamycin and intermediate to erythromycin (63.63%) and norfloxacin (63.63%) whereas 100% isolates were resistant to amoxicillin. The isolates of *E. coli* showed 100%, 80% and 60% resistant to amoxicillin, norfloxacin and erythromycin respectively whereas 100%, 80% and 60% isolates were found sensitive to azithromycin, tetracycline, gentamycin and ciprofloxacin (table 2). In this study *Vibrio* spp., *E. coli* and *Staphylococcus* spp. were found multidrug resistant (resistant against 2-3 antibiotics). The results of antibiotic susceptibility test of the bacterial isolates of current study are closely correlated to Hossain *et al.* [12], reported 100% isolated of *E. coli* showed resistant to amoxicillin. In case of *Vibrio* spp. similar result was observed by Mahbub *et al.* [19], reported highest resistance to amoxicillin. Ferrini *et al.* [21] reported that 82% of *Vibrio* isolated from seafood showed resistance to ampicillin. The results of *Staphylococcus* spp. agrees with the results of Onanuga and Temedie [22], showed higher resistance to amoxicillin and susceptible to gentamycin. Data of this study suggests that shrimp samples of Khulna region contaminated with multidrug resistant foodborne bacteria might pose public health hazard.

Table 2: Results of antibiotic susceptibility test of isolated bacteria

Name of antibiotic	*Staphylococcus* spp. (n=11)			*E. coli* (n=5)			*Vibrio* spp. (n=10)		
	S	I	R	S	I	R	S	I	R
CIP (5µg)	63.63%	36.36%	0	60%	40%	0	70%	30%	0
S (10µg)	54.54%	36.36%	9.09%	20%	20%	60%	50%	50%	0
GEN (10µg)	72.72%	27.27%	0	80%	20%	0	80%	20%	0
AMX (25µg)	0	0	100%	0	0	100%	0	0	100%
TE (30µg)	81.81%	18.18%	0	80%	20%	0	60%	0	40%
E (5µg)	27.27%	63.63%	9.09%	0	40%	60%	40%	60%	0
AZM (30µg)	90.90%	9.09%	0	100%	0	0	70%	30%	0
NOR (10 µg)	27.27%	63.63%	9.09%	20%	0	80%	10%	60%	30%

Legends: S= Sensitive, I= Intermediate, R= Resistant, CIP= Ciprofloxacin, S= Streptomycin, GEN= Gentamycin, AMP= Amoxicillin, TE= Tetracycline, E= Erythromycin, AZM= Azithromycin and NOR= Norfloxacin

2.4 Prevalence of bacteria

The estimated proportionate prevalence of isolated *Vibrio* spp., *Staphylococcus* spp. and *E. coli* from shrimp samples were 26.67%, 40% and 26.67% respectively in Rupsha and 40%, 33.33% and 6.67% respectively in Mongla, Khulna (fig 4). Similar types of bacteria were isolated by previous authors from shrimp, seafood and other fish sample [12, 23-34]. These results uncover that shrimp of this two processing plant had great exposure to foodborne microorganism. These organisms might enter into the processing plant in different stages starting from farming area (pond/gher), hatcheries, collection center, transportation and receiving area. Presence of these foodborne pathogens reduces the quality of shrimp and also decreases the export value. Personal hygiene of working personnel and their poor sanitary practices enhance the bacterial contamination in shrimp.

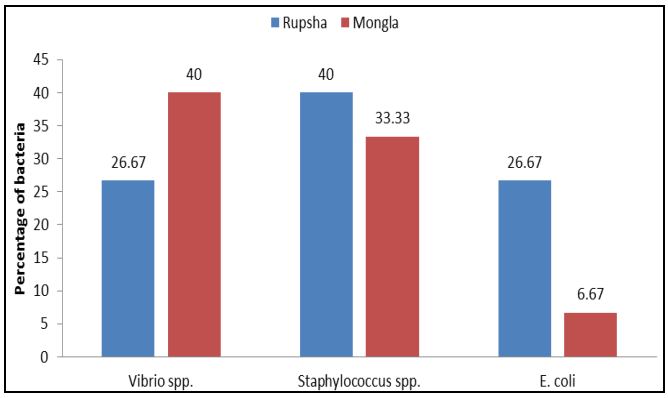

Fig 4: Prevalence of different bacteria collected from Khulna region (Rupsha and Mongla)

4. Conclusion

Considering the findings of this research work, it may be concluded that *Vibrio* spp.; *Staphylococcus* spp. and *E. coli* were successfully isolated and confirmed by different cultural media, biochemical tests. Isolated bacteria showing the multidrug resistance for commonly used antibiotics are alarming. The concentrations of bacteria in shrimp of Rupsha were lower than the shrimp of Mongla. Presence of *E. coli* in

shrimp indicated the maintenance of low hygienic condition during processing. So, the processing of shrimp must be performed in a standard hygienic way from the onset of the handling to minimize the incidence of microbial contamination and effect of multidrug resistance on human health.

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