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Microbiological quality analysis of shrimps collected from local market around Dhaka city

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Abstract

Shrimps sold in local markets could be microbiologically spoiled intrinsically or extrinsically. Present study attempted to detect the frequency of such microorganisms in shrimps collected from local markets of Dhaka city. A total of 7 categories of shrimp samples were studied. All of them were found to be contaminated with *Staphylococcus* spp., *Aeromonas* spp., *Klebsiella* spp., *Pseudomonas* spp. and *Shigella* spp. ranged from 1.5×10^4 to.7.9×10^8 cfu/g with a comparatively higher frequency of *Klebsiella* spp., *Staphylococcus* spp., and *Aeromonas* spp. Study of antibiogram revealed multi-drug resistance of most of the isolates. No antimicrobial activity was detected.

Introduction

Shrimps deteriorate due to improper handling, and further processing can never bring back its freshness. Low quality frozen foods are related with improper processing and poor hygienic conditions. Contamination in shrimp may be due to poor hygienic condition including inappropriate processing, preservation and storage condition (Frazier and Westhoff, 1995; Dalsgaard *et al.*, 1995; Huss, 2003; Eze *et al*., 2010). Consequently, shrimps may be contaminated with different types of bacteria such as *Vibrio* spp., *Salmonella* spp., coliform, fecal coliform, streptococci and Staphylococcus spp., those spoil fishes and are responsible for causing cholera and other food borne disease outbreaks (Snowdon *et al.*, 1989; Starutch, 1991; Karunasagar *et al*., 1994; Cray and Moon, 1995; Wallace *et al*., 1999; Mobin *et al*., 2001; WHO, 2012).

Nowadays, shrimp plays a dominant role in the economy of Bangladesh. Every year it contributes 4.7% to GDP and about 8% to the total export earnings of the country. Therefore, by considering the consumer health safety and economical sustain it is worth to maintain the microbiological quality of the fish.

Drug resistance virulent genes of the spoiling micro flora are another important concern on the shrimp cultivation and consumption which may possess serious health threat especially in case of disease medication (Tenover, 2006; Bennett, 2008; Canton, 2009; Hung and Kaufman, 2010). On the contrary, shrimps could possess the antimicrobial activity as well, depending on the composition of the polysaccharide chitin (Wang *et al*., 1999; Varadharajan *et al.*, 2012).

Along these lines, the present investigation attempted to quantify the pathogenic bacteria in the local shrimp samples, to demonstrate the drug-resistance traits of the isolates, and finally to detect the anti-bacterial activity (if any) of the studied shrimp samples.

Materials and Methods

Study area, sampling and sample processing

Total 7 categories of shrimp samples such as category I: *Palaemon Karnafuliensis* (*Karnafuli chingri* or *Gura icha*), category II: *Penaeus Orientalis* (*Chapda chingri*), category III: *Metapenaeus affinis* (*Kerani chingri*), category IV: *Metapenaeus dobsoni* (*Gosha chingri*), category V: *Parapenaeopsi uncta* (*Khoira chingri*), category VI: *Solenocera indica* (*Kada chingri*) and category VII: *Alphaeus euphrosyne* (*Pina icha*) were collected randomly from different local market in Dhaka city within a time frame of October, 2012 to January, 2013. Ten Samples of each category were collected aseptically early in the morning and transported immediately to the laboratory using sterile polyethylene bags with ice. At first, the shrimp samples were divided into three parts - head, body and tail. Then 10 g of all part of the samples were homogenized through blending with 90 ml peptone water individually in sterile automatic blender and were serially diluted up to 10^{-6}

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(Cappuccino and Sherman, 1996).

Microbiological analysis

Estimation of total viable bacteria (TVB)

The sample (0.1 ml) was spread onto nutrient agar (NA) for enumerating total viable bacteria (TVB). After spreading, the plates were incubated at 37°C for 24 hours.

Isolation of Salmonella spp. Shigella spp. and Pseudomonas spp.

From the homogenized samples, 1 ml of sample was transferred to 9 ml of selenite cysteine broth and alkaline peptone water (10^{-1} dilution) for the enrichment of *Salmonella* spp. and *Vibrio* spp., respectively, which were incubated at 37°C for 6 hours. The enriched sample was then subjected to 10-fold serial dilution up to 10^{-4}. The sample (0.1 ml) was spread onto Salmonella Shigella (SS) agar and thiosulphate citrate bile salt sucrose (TCBS) agar plates from 10^{-2} and 10^{-4} dilution tubes. The samples were incubated at 37°C for 24 hours to determine the typical colony characteristics.

Isolation of Escherichia coli and Klebsiella spp.

Escherichia coli and *Klebsiella* spp. were isolated by spreading 0.1 ml of sample from 10^{-2} and 10^{-5} dilution tubes on to the surface of MacConkey agar medium and was incubated at 37°C for 24 hours. After incubation, the plates were observed and the presence of *E. coli* was further confirmed by using the eosin-methylene blue (EMB) agar medium which was indicated by bluish-black colony with green metallic sheen.

Isolation of Staphylococcus spp., Pseudomonas spp., and Listeria spp.

For the isolation of *Staphylococcus*, *Pseudomonas* and *Listeria* spp., 0.1 ml of diluted sample was spread onto mannitol salt agar (MSA), *Pseudomonas* agar and *Listeria* identification media, consecutively and all the plates were incubated at 37°C for 24 hours.

All the suspected isolates were biochemically examined by following standard protocol for the further confirmation of the presence of pathogenic bacteria (Cappuccino and Sherman, 1996; Alfrad, 2007).

Determination of drug susceptibility pattern

The pathogenic isolates were examined for antibiotic susceptibility traits (either drug resistant or sensitive) by disc diffusion assay on Mueller-Hinton agar (Difco, Detroit, MI) against commonly used antibiotics following the standard protocol (Bauer *et al.*, 1966; Ferraro, 2001, Munshi *et al*., 2012; Acharjee *et al*., 2013). Antibiotic discs such as trimethoprime/sulfamethoxazole (25 µg), erythromycin (15 µg), amoxicillin (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), streptomycin (10 µg), ampicillin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), cefixime (5 µg), polymixin B (300 units), kanamycin (30 µg), vancomycine (30 µg), gentamycine (10 µg), nalidixic acid (30 µg), azythromycine (15 µg) and penicillinG (10 µg) were used.

Determination of antibacterial activity in the shrimp

The investigation of the antibacterial activity of the shrimp samples was performed by using agar well diffusion method (Jagessar *et al*., 2008; Hussain *et al*., 2010) and also by spot test. In agar well diffusion method, pathogens (*Pseudomonas* spp, *Listeria* spp, *Aeromonas* spp, *Vibrio* spp, *Salmonella* spp, *Klebsiella* spp, *Staphylococcus aureus* and *E. coli*) were spread over the entire surface of MHA and wells were made by cork borer. Then, the blended sample was added along with a positive control (antibiotic disc) and a negative control (normal saline) into the wells. The presence of antimicrobial activity was indicated by the production of clear zone around the wells. Spot test was performed by dropping blend of different part of shrimp at different concentrations (10, 20, 40 and 100 µl) on MHA and were allowed to dry off.

Statistical analysis

All the experiments were performed in triplicate. Statistical analyses were performed by determining the p-value through t test.

Results and Discussion

By considering the public health significance, present study endeavored to emphasis the total microbial array, drug resistance trait and the antimicrobial activity of the shrimp samples.

Prevalence of pathogenic microorganisms

All the categories of samples exhibited higher microbial loads within a range of 1.5×10^4 cfu/g to 3.3×10^8 cfu/g (Table 1). The total aerobic bacterial load was found to be higher (1.2×10^4 cfu/g to 3.3×10^4 cfu/g) in all parts of shrimp samples. The load of *Staphaylococcus* spp. were detected within a range of 1×10^6 cfu/g to 2×10^7 cfu/g, 2.7×10^5 cfu/g to 2.3×10^7 cfu/g, and 1.3×10^6 cfu/g to 2.3×10^7 cfu/g in head, body and tail of shrimp samples, consecutively. Whereas, *Klebseilla* spp. were encountered within a range of 1.6×10^5 cfu/g to 2.4×10^7 cfu/g, 2.8×10^5 cfu/g to 1.5×10^7 cfu/g, and 2.5×10^5 cfu/g to 2.3×10^7 cfu/g in head, body and tail, consecutively. *Aeromonas* spp.

Table 1. Bacterial load (cfu/g) of the local shrimp sample

Samples	TVB	[1]*Shigella* spp.	*Staphylococcus* spp.	*Klebsiella* spp.	*Aeromonas* spp.	*Pseudomonas* spp
Category I n=10						
Head	1.4×10^8	0	2.1×10^6	1.9×10^6	3.1×10^7	0
Body	1.3×10^8	0	1.7×10^6	-	5.6×10^6	0
Tail	1.3×10^7	0	2.3×10^7	4.9×10^5	2.9×10^7	0
Category II n=10						
Head	2.3×10^7	0	1.3×10^6	2.4×10^7	2.2×10^5	1.6×10^6
Body	1.2×10^6	0	1.5×10^6	2.3×10^6	1.6×10^6	1.4×10^7
Tail	1.5×10^8	0	2.3×10^6	2.6×10^6	0	2.2×10^5
Category III n=10						
Head	1.5×10^8	1.4×10^7	1.7×10^7	2.9×10^6	1.4×10^5	2.4×10^7
Body	1.7×10^7	0	2.3×10^7	2.8×10^5	1.5×10^4	1.0×10^7
Tail	1.4×10^7	1.8×10^6	1.3×10^6	1.9×10^6	0	7.9×10^7
Category IV n=10						
Head	2.3×10^8	0	2.8×10^6	1.7×10^6	1.6×10^6	0
Body	3.3×10^7	0	1.3×10^6	1.5×10^7	2.2×10^6	0
Tail	1.5×10^8	0	2.0×10^6	2.3×10^7	2.4×10^5	0
Category V n=10						
Head	3.3×10^8	0	1.0×10^6	1.6×10^7	2.2×10^5	0
Body	2.3×10^7	0	2.7×10^5	2.5×10^6	2.0×10^6	0
Tail	1.7×10^7	0	1.6×10^7	2.6×10^5	1.4×10^5	1.7×10^6
Category VI n=10						
Head	2.7×10^8	0	2.0×10^6	1.6×10^5	2.8×10^6	1.3×10^6
Body	1.3×10^7	0	1.7×10^6	2.6×10^6	1.7×10^7	0
Tail	2.0×10^8	1.7×10^6	1.8×10^7	2.5×10^5	2.8×10^7	0
Category VII n=10						
Head	3.5×10^7	0	2.0×10^7	2.4×10^5	1.8×10^5	2.0×10^7
Body	5.3×10^7	0	2.2×10^6	2.0×10^6	2.2×10^7	2.2×10^6
Tail	1.3×10^8	0	1.6×10^6	1.6×10^6	2.0×10^6	2.7×10^7

E. coli and *V. cholerae* were absent in all the samples. Average counts (cfu/g) from all samples have been shown here.
[1]Bacterial load after enrichment (Prior to enrichment, the recovery was nil).
All the experiments have been done three times and the results were reproducible. One representative data have been shown. All data were found to be significant ($p < 0.1$).

Table 2. Results of biochemical tests of the pathogenic isolates from shrimp sample

Number of isolates	TSI				Motility	Indole Production	MR	VP	Citrate utilization	Oxidase	Identified microorganism
	Slant	Butt	Gas	H_2S							
1	R	A	+	+	-	+/-	+	-	-	ND	*Shigella* spp.
2	A	K	+	+	+	-	+	-	-	-	*Staphylococcus* spp.
3	A	A	+	-	-	-	-	+	+	-	*Klebsiella* spp.
4	R	A	-	-	+	-	+	+	+	-	*Aeromonas* spp.
5	R	R	-	-	+	-	-	-	+	+	*Pseudomonas* spp.

TSI = Triple Sugar Iron Test, Y = Yellow (Acid), R = Red (Alkaline), MR = Methyl red, VP = Voges-Proskauer, ND = Not Done

($\geq 10^7$ cfu/g) were found in all parts of category one, four, five, six and seven shrimp samples. The tail of category two and three were free from *Aeromonas* spp., while the head and body of both categories were found to be contaminated with *Aeromonas* spp. ranged from 1.5×10^4 cfu/g to 1.6×10^6 cfu/g. The load of *Shiegella* spp. was totally absent in all parts of category one, two, four, five and seven samples. Only category three and six samples were contaminated with *Shigella* spp. within a range of 1.7×10^6 cfu/g to 1.4×10^7 cfu/g. The load of *Pseudomonas* spp. was found to be nil in all parts of category one and four samples. On the other hand, *Pseudomonas* spp. was found within the range 2.2×10^5 cfu/g to 7.9×10^7 cfu/g in category two, three, five and seven samples (Table 1). *E. coli* and *V. cholerae* were absent in all the samples. All the isolates were biochemically identified (Table 2).

The pathogenic profile in this study confer that the overall quality of the shrimp samples was not satisfactory. In most of the cases, the pathogenic load exceeded safety limit (ICMSF, 1986) which might

Table 3. Antibiogram of the pathogenic isolates

	Pathogens									
	Shigella spp. n=25		*Klebsiella* spp. n=56		*Pseudomonas* spp. n=38		*Aeromonas* spp. n=64		*Staphylococcus* spp. n=67	
Antibiotics	R	S	R	S	R	S	R	S	R	S
AMP	69%	31%	75%	25%	80%	20%	99%	1%	90%	10%
CIP	10%	90%	80%	20%	10%	90%	68%	32%	ND	ND
PIP	ND	ND	ND	ND	ND	ND	100%	0%	90%	10%
CEF	12%	88%	30%	70%	70%	30%	ND	ND	ND	ND
AMO	27%	73%	10%	90%	ND	ND	90%	10%	80%	20%
IPM	10%	90%	15%	85%	70%	30%	ND	ND	ND	ND
CHL	58%	42%	40%	60%	30%	70%	35%	65%	ND	ND
TMP-SUL	12%	88%	15%	85%	70%	30%	78%	22%	30%	70%
GEN	0%	100%	30%	70%	ND	ND	15%	85%	35%	75%
NALI	100%	0%	10%	90%	85%	15%	ND	ND	ND	ND

All the experiments have been done three times and the results were reproducible. One representative data have been shown.
ND = Not done, N = Number of isolates, R = Resistant, S = Sensitive, AMP = Ampicillin 10 µg, NALI = Nalidixic Acid 30 µg, N = Number of isolates, AMO = Amoxicillin 10 µg, IPM = Imipenem 30 µg, CIP = Ciprofloxacin 5 µg, CHL = Chloramphenicol 10 µg, CEF = Ceftriazone 30 µg, PIP = Piperaciline 10 µg, GEN = Gentamycin 10 µg, TMP/SUL = Trimethoprime- sulfomethoxazole 25 µg.

have a great effect on overall public health. The routes of contamination might be unhygienic handling, contaminated water source, improper packaging, transportation and storage (Antony *et al.*, 2002).

Frequency of drug-resistant isolates

Occurrence of drug resistance genes in pathogenic isolates is becoming a serious problem in developing countries where antibiotic misuse is very common. Such drug resistance of the pathogens also be a great threat for the treatment of the diseases, even in the developed countries (Gubala and Proll, 2006; Bhatta *et al.*, 2007; Jakee *et al*., 2009). Several mechanical, epidemiologic and genetic factors may lead to the development of drug resistance (Bennett, 2008; Canton, 2009; Hung and Kaufman, 2010) The study of antibiogram revealed that the most of the pathogens were found to be resistant against commonly used antibiotics including ampicillin, ciprofloxacin, amoxicillin, chloramphenicol, trimethoprime-sulfomethoxazole, while sensitive against imipenem, piperaciline, nalidixic acid, gentamycin and ceftriazone (Table 3).

Presence of antimicrobial activity of the shrimp

Several studies worldwide and in Bangladesh reported antimicrobial activity in different food samples (Kyung *et al*., 1994; Dubey *et al*., 2010; Hussain *et al.*, 2010). One of our previous studies was conducted to establish the antimicrobial activity of export quality shrimp samples in Bangladesh (Rahman *et al*., 2012). The present study showed the absence antimicrobial activity in the local shrimp samples. The shrimp samples might not be previously processed with any antimicrobial agent during storage and before transported to the market.

Conclusion

Overall, the present study investigated the shrimps of local markets which had been found to harbor a huge array of pathogenic microorganisms. Presence of multidrug resistance traits among the isolates also accelerated the public health threat. Considering these findings, the present study suggested to follow a proper guideline for the maintenance of microbiological quality of shrimps. Proper hygiene and sanitation should be maintained throughout the time period between capture and delivery to the consumers of the shrimps which thereby aid in the reduction of food borne disease outbreaks.

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