Microbiological Conditions of Frozen Shrimp in Different Food Market of Dhaka City

F. R. PINU¹, Sabina YEASMIN², Md. Latiful BARI^{3*} and M. M. RAHMAN¹

¹ Department of Microbiology, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka 1217, Bangladesh

² Department of Genetic Engineering and Biotechnology Dhaka University, Dhaka-1000, Bangladesh

³ Food Hygiene Laboratory, National Food Research Institute, 2-1-12, Kannondai, Tsukuba-shi, Ibaraki 305-8642, Japan

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This study was carried out to evaluate the microbiological condition of the frozen shrimps found in the local markets and departmental chain shops of Dhaka city. Pathogenic bacterial load were found greater in the samples of departmental shops rather than that of local markets. The highest Aerobic Plate Count (APC) found in the samples of departmental shops and local markets were $8.74 \log_{10}$ CFU/g (sample No. 14) and $8.30 \log_{10}$ CFU/g (sample No. 8), respectively. However, the lowest APC found in the samples of departmental shops and local markets were $5.73 \log_{10}$ CFU/g (sample No. 5) and $6.44 \log_{10}$ CFU/g (sample No. 12), respectively. Presence of *Vibrio* spp. was confirmed in 8 samples (5 from departmental shops and 3 from local markets). It was confirmed that 8 samples contained *Shigella* spp and 11 samples had *Salmonella* spp. Antibiotic sensitivity pattern of the indicator and pathogenic isolates was determined and nearly all of them were resistant to penicillin and bacitracin. Most of the isolates were sensitive against tetracycline, kanamycin and chloramphenicol. It was observed from the study that the samples collected from local markets and departmental shops were heavily contaminated and are of special concern for human consumption.

Keywords: Microbiology, Frozen Shrimp, Food Market

Introduction

Both marine and fresh water fish are natural resources in Bangladesh, which cater to the enormous need of protein and other nitrogenous constituents (14). The economic potential of the marine fisheries sector in Bangladesh is considered to be enormous in recognition of the country's 710 kilometers coastline and Exclusive Economic Zone, which spreads over an area of 164,000 square kilometers. According to Bangladesh Statistical Year Book, Bangladesh exported 26.56 million tons shrimp during last fiscal year 2003–2004 (5).

The major part of the shrimps exported from Bangladesh is destined for USA (31.8%), Japan (18.3%), Belgium (16.2%) and U.K. (11%). Shrimp culture in Bangladesh is predominantly carried out by non-local entrepreneurs on leased-in lands (4).

Microbiological condition of the shrimp is mainly depended on the condition of transport, handling and processing. Frozen shrimps are often contaminated after catching during the above-mentioned steps (17). The water used to wash shrimp may be contaminated heavily with spoilage organisms and pathogens that can grow on the shrimp. Proper handling of shrimp between harvesting and marketing to the consumer is a crucial element in assuring quality of the final product. Standards of sanitation, method of handling and the time/temperature of holding shrimp are all significant factors to assure quality

(20). With a few exceptions, fish are considered free of pathogenic bacteria of public health significance when first caught. The presence of bacteria harmful to humans generally indicates poor sanitation in handling and processing, and the contamination is almost always of human or animal origin.

The objective of this study was to determine the microbiological status of frozen shrimps in departmental shops and local markets of Dhaka city. Specific objectives are: 1) presence of pathogen in frozen shrimp in the local markets, 2) presence of indicator organisms of fecal pollution (coliforms, *Escherichia coli*) 3) or other types of pathogens or indicator organisms 4) indicator organisms if any and their antibiotic sensitivities were also examined.

Materials and Methods

Collection of shrimp samples Shrimp samples were collected from different departmental shops and local retail shops at Dhaka city in between August 2005 to February 2006. Total twenty samples were collected and analyzed for the microbiological status of the shrimp sample. Among the 20 samples, 10 were collected from departmental chain shops and 10 were from local markets. The shrimp samples were transported to the laboratory in an insulated box with ice to maintain the temperature (4 to 6 °C) and stored at -18°C at the laboratory until use. Special sterile Ziploc bags were used for carrying/thawing all the samples to avoid further contamination. Samples were processed and used within 24 hours of collection.

Processing of the samples The samples were thawed

* To whom correspondence should be addressed.

E-mail: latiful@affrc.go.jp

at room temperature for 5–6 h to melt the ice and were taken to the safety cabinet to peel off the shell and removing the head. The edible part body was taken and made small pieces using sterile scissors. Twenty five (25) grams of shrimp sample was measured carefully and accurately by using a weighing machine and dissolved in 225 mL of physiological saline (0.85% NaCl solution) in a stomacher bag. Each sample was then stomached for 60 seconds separately. One milliliter/0.1 mL of the stomacher-treated samples was then pour or spread plated in the microbiological medium as describe below:

Microbiological analysis The microbiological analysis was performed as per the standard methods adopted from Online Bacteriological Analytical Manual, USFDA for detection, enumeration and identification of individual organisms (3).

Aerobic plate count (APC) After appropriate serial dilutions, the samples were pour-plated on nutrient agar (Becton Dickinson, France). The colonies were counted after 24 h of incubation at 35–37°C.

Coliform count Dilutions made for APC were pour-plated on MacConkey agar (Oxoid Ltd., Hampshire, England); Typical pink colonies were counted after 24 h of incubation at 35–37°C.

Fecal coliform and *E. coli* Dilutions made for APC were pour-plated on MacConkey agar (Oxoid Ltd., Hampshire, England). Typical colonies were counted after 24 h of incubation at 35–37°C. The suspected *E. coli* isolates were streaked on mFC agar plates and incubated at 44.5°C for 24 h. Typical blue colonies were counted and was further confirmed by growing in eosin methylene blue (EMB) agar plates (Oxoid Ltd., Hampshire, England).

***Staphylococci* count** Dilutions made for APC were spread-plated on mannitol salt agar (MSA) (Oxoid Ltd., Hampshire, England). Typical yellow colonies were counted after 48 h of incubation at 35°C.

Salmonella The sample (25 g) was homogenized in selenite cysteine broth and incubated overnight at 37°C. After the samples were enriched a loopful of growth from the broth was streaked on Salmonella-Shigella agar (SSA; Oxoid), typical black colonies from plates were isolated and identified by biochemical tests.

Shigella spp. The sample (25 g) was homogenized in 225 mL of sterile physiological saline. After appropriate serial dilutions, the samples were pour-plated on xylose lysine deoxycholate (XLD) agar (Oxoid Ltd., Hampshire, England). Typical pink and translucent colonies with or without rough edges were picked up after 48 h of incubation at 35–37°C and were confirmed by biochemical tests.

Vibrio spp. The sample (25 g) was homogenized in 225 mL of alkaline peptone water (APW) and incubated at 35°C for 24 h. After appropriate serial dilutions, the homogenates were pour-plated on TCBS agar (Oxoid Ltd., Hampshire, England). The suspected *Vibrio* isolates were then grown in different NaCl concentration as 3, 5, 10 and 15% and were confirmed by biochemical tests.

Biochemical tests Biochemical tests were done according to the manual for general bacteriology of the American Society of Microbiology (2). Biochemical tests

done were as follows: oxidase test, catalase test, carbohydrate fermentation/ utilization test, Kligler's iron agar (KIA) test, indole production test, methyl red (MR) test, Voges-Proskauer (VP) test, citrate utilization test, nitrate reduction test, motility indole urea (MIU) tests, and salt tolerance (3%, 5%, 10% and 15% NaCl) test were performed to identify the bacteria of interest (7).

Determination of antibiotic susceptibility of the isolated pathogens from shrimp samples Susceptibility of *E. coli*, *Salmonella*, *Shigella*, *Vibrio* and *Staphylococcus* isolates to different antimicrobial agents was measured *in vitro* according to the Kirby-Bauer methods (5). Commercially available antimicrobial discs [ampicillin (AMP), amoxicillin (AML) bacitracin (B), chloramphenicol (C), ciprofloxacin (CIP), erythromycin (E), gentamycin (GN), penicillin G (PG), streptomycin (S), tetracycline (TE), penicillin (P) and kanamycin (K)] were used for the test. The *E. coli*, *Salmonella*, *Shigella*, *Vibrio* and *Staphylococcus* isolates were used against the antibiotics.

Results and Discussion

Aerobic plate count (APC) of the frozen shrimp samples Estimation of bacterial numbers in shrimp is frequently used to retrospectively assess microbiological quality or to assess the presumptive safety of the product. The APC of all the samples were found high and cross the limit given by International Commission on Microbiological Specifications for Foods (ICMSF). The high microbial load could deteriorate the product quality within short time. The highest load was found in sample 14 (8.74 log₁₀ CFU/g) and the lowest load was in sample 7 (5.17 log₁₀ CFU/g) (Table 1 and 2). This might be because of the contamination from the source of water, poor hygiene and sanitation condition of the processing premises (18). Usually in Bangladesh, in the ghers or other catching side shrimps are collected in boats or trawlers. The surfaces of these places are contaminated due to lack of proper washing and inadequate disinfections. Catching vessels also act as source of contamination of harmful microorganisms (17). As a result many microbes begin to multiply before freezing. But freezing only can limit the growth of microbes for a short period. When favorable condition comes back, most of the microbes can multiply within short time (16).

Coliform bacteria Total coliforms were present in all the samples in high number. According to ICMSF limits,

Table 1. Average population of aerobic mesophilic, coliform and fecal coliform counts in shrimp samples of local markets.

Sample No	Source	Population log ₁₀ CFU/g:		
		Aerobic mesophilic bacteria	Total coliform bacteria	Fecal coliform /E.coli
1	Local	7.41	5.81	1.0
2	Local	7.47	5.92	ND
8	Local	8.30	4.50	2.0
9	Local	6.65	3.36	0.9
12	Local	6.44	3.46	1.08
13	Local	7.60	4.64	2.15
16	Local	6.62	2.70	ND
17	Local	7.68	3.72	ND
18	Local	7.55	3.39	1.34
19	Local	6.61	5.85	1.56 /+Ve
Average population, 7.23 log ₁₀ CFU/g, Standard Deviation, 0.58, n=10, ND, not detected				

Table 2. Average population of aerobic mesophilic, coliform and fecal coliform counts in shrimp samples of departmental shop.

Sample No	Source	Population log ₁₀ CFU/g:		
		Aerobic mesophilic bacteria	Total coliform bacteria	Fecal coliform /E.coli
3	Dept.	6.08	4.70	ND
4	Dept.	6.90	4.76	1.69
5	Dept.	5.73	5.73	0.60
6	Dept.	7.95	4.89	ND
7	Dept.	5.17	5.71	2.34 / +Ve
10	Dept.	7.08	3.41	ND
11	Dept.	8.53	3.70	ND
14	Dept.	8.74	3.15	2.47
15	Dept.	5.50	2.70	ND
20	Dept.	5.77	4.25	1.30
Average population, 6.71 log ₁₀ CFU/g, Standard Deviation, 1.18, n=10, ND, not detected				

2.0 log₁₀ CFU/g can be present in the seafood. All the sample exceeded this limit. The highest load was 5.92 log₁₀ CFU/g in sample 2 and the lowest load was 2.70 log₁₀ CFU/g of sample 14 and 15 (Table 1 and 2). The presence of total coliform suggests the unhygienic condition or not properly handled of the shrimp samples including holding temperature. Moreover, the contamination may also come from wash water or water use for icing (10). Therefore, it is necessary to use good quality water to avoid contamination.

Fecal coliform and *E. coli* Fecal coliform like *E. coli* are the group of the total coliforms that are considered to be present specifically in the gut and feces of warm-blooded animals. Because the origins of fecal coliforms are more specific than the origins of the total coliform group of bacteria, fecal coliforms are considered a more accurate indication of animal or human waste than the total coliforms (8). About 80% of the shrimp samples was found contaminated with the fecal coliform; 20% of them was found contaminated with *E. coli* (Table 1 and 2). This result indicates that the water or processing vessels are somehow contaminated fecally. Despite the fact that they cannot be linked directly to contamination by human sewage, since this bacteria found in high concentration within sewage/septic systems. However, the presence of *E. coli* is not permitted in the shrimp samples in Japan, USA and other European countries. (21)

Load of pathogenic bacteria The presence of total and fecal coliform suggested that other harmful and pathogenic microorganisms such as *Salmonella*, *Shigella*, *Vibrio* spp. and *Staphylococcus aureus* are present in the samples. For that reason, further identification procedure was carried out to find out the pathogens.

Salmonella *Salmonella* are usually transmitted to humans by eating foods contaminated with animal feces. Contaminated foods are often of animal origin, such as beef, poultry, milk, or eggs, but all foods, including vegetables may become contaminated. Many raw foods of animal origin are frequently contaminated, but fortunately, though cooking kills *Salmonella*. The unwashed hands of an infected food handler, who forgot to wash his or her hands with soap after using the bathroom, may also contaminate food (9). *Salmonella* was identified in 11 samples out of 20 (Table 3).

A recent review by Reilly *et al.* (19), presented the evidence that farmed tropical shrimps frequently contain

Table 3. Presence of *Salmonella*, *Shigella*, *Vibrio* spp. and *Staphylococcus* spp. in different shrimp samples.

Sample No	Population log ₁₀ CFU/g:			
	<i>Salmonella</i> spp.	<i>Shigella</i> spp.	<i>Vibrio</i> spp.	<i>Staphylococcus</i> spp.
1	2.34	2.04	1.30	5.63
2	ND ^a	1.47	1.47	4.50
3	1.30	ND	1.0	3.64
4	2.30	2.11	0.90	5.43
5	ND	ND	ND	3.77
6	ND	1.41	ND	2.85
7	2.0	ND	1.44	4.51
8	ND	ND	ND	4.51
9	1.50	1.34	ND	2.96
10	ND	ND	1.08	3.56
11	ND	ND	ND	4.64
12	1.68	ND	ND	2.34
13	1.41	ND	ND	3.73
14	1.14	1.36	ND	4.47
15	ND	ND	1.38	3.04
16	ND	ND	ND	3.41
17	1.11	1.56	ND	3.57
18	ND	ND	ND	4.69
19	1.3	1.17	0.69	2.47
20	1.0	ND	ND	4.79

^aND, not detected.

Salmonella. It has also been demonstrated that *Salmonella* in aquaculture shrimp products originates from the environment rather than from poor standards of hygiene, sanitation, and poultry manure as feed. Most shrimps are cooked prior to consumption. These products, therefore, pose minimal health risks to the consumers except for cross contamination in the kitchens (11).

***Shigella* spp.** All *Shigella* spp. have been implicated in foodborne outbreaks, however, *S. sonnei* is clearly the leading cause of shigellosis from food. *Shigella* are present in the diarrheal stools of infected persons while they are sick and for a week or two afterwards (22). *Shigella* infections may be acquired from eating contaminated food. Infected food handlers who forget to wash their hands with soap after using the bathroom may contaminate food. In this study, 8 samples were found to be contaminated with *Shigella* spp (Table 3).

***Vibrio* spp.** In case of *Vibrio* spp., eight samples were found to be containing this bacterial species. The highest load was found in sample 2 (1.47 log₁₀ CFU/g) (Table 3). It is very unusual to find *Vibrio* spp. in frozen samples because *Vibrio* normally cannot survive in the frozen samples and it requires moisture to remain alive (13). The reason of survival of *Vibrio* spp in frozen shrimp samples was probably the inadequate freezing condition and presence of moisture during freezing.

However, no *Vibrio cholera* and *Vibrio parahaemolyticus* were found in all the sample tested.

***Staphylococcus* spp.** Seafood may be contaminated with *Staphylococcus* via infected food handlers or from the environment. More often the contamination is from an infected individual with an infection on hands or with a cold or sore throat. Staphylococci are poor competitors and do not grow well in the presence of other microorganisms (1). Thus, the presence of staphylococci in raw, naturally contaminated food is of little significance. In contrast, rapid growth and toxin production can take place in precooked seafood (shrimp) if recontaminated with *S. aureus* produces a large amounts of enterotoxin, when growing in the food. These toxins are generally

Table 4. Antibiotic sensitivity of *E. coli*, *Salmonella*, *Shigella* and *Staphylococcus* isolates.

Isolated strains	Resistant	Intermediate	Sensitive
<i>E. coli</i> -1	P, AMP, E, B, AML	PG	C, K, S, TE
<i>E. coli</i> -2	P, B, E, PG	AMP, TE	K, CN, S, AML
<i>E. coli</i> -3	E, PG, B, P	AMP	K, C, CN, S, TE
<i>E. coli</i> -4	P, B, E	C, CN, AML	K, S, TE, PG
<i>E. coli</i> -5	E, PG, CN, TE, S, P	AMP	K, S, B
<i>Salmonella</i> -1	P, B	AMP, AML	PG, TE, K, C, CN, S
<i>Salmonella</i> -2	P, PG, B	AMP	AMP, K, C, CN, AML, TE
<i>Shigella</i>	AML, PG, P, B, TE, C	AMP, E	CN, K
<i>Staphylococcus</i> -1	AMP, AML, P, B, TE	Nil	C, S, PG, K, E
<i>Staphylococcus</i> -2	AML, AMP, B	TE, K, E	S, K, PG, P

Abbreviations: Ampicillin (AMP), Amoxicillin (AML), Bacitracin (B), Chloramphenicol (C), Ciprofloxacin (CN), Erythromycin (E), Gentamycin (GN), Penicillin G (PG), Streptomycin (S), Tetracycline (TE), Penicillin (P) and Kanamycin (K).

very resistant to proteolytic enzymes and heat. The heat applied in pasteurization and normal household cooking is not sufficient to destroy the toxin (18). There are considerable numbers of *Staphylococcus* spp. found in almost all the sample tested (Table 3). However, there were no *S. aureus* in the samples tested.

Antibiotic sensitivity pattern of *E. coli*, *Shigella*, *Salmonella* and *Staphylococcus* isolates Ten isolates (five *E. coli*, two *Salmonella*, one *Shigella* and two *Staphylococcus* spp.) and twelve antibiotics (AMP, AML, B, C, CIP, E, GN, PG, S, TE, P and K) were used for the sensitivity test. It was found that all isolates were resistant against PG (Table 4). Among the five isolates of *E. coli*, four were resistant to PG, B and E. *Salmonella* and *Shigella* isolates were also sensitive to PG and B. Both *Staphylococcus* isolates were resistant to AML, AMP, B and one isolate was found resistant to TE. The uncontrolled and irresponsible use of antibiotics and other chemotherapeutic agents are responsible for the occurrence of the antibiotic resistant trait among the pathogens and the majority of the drug resistant bacteria carry drug resistant (R) factor (15). It was interesting to observe some antibiotic sensitive strains in this study.

Conclusion

Our study demonstrated that the levels of bacteria in the frozen shrimp were found higher than the limit offered by ICMSF. The total aerobic bacteria, and coliform count was found higher which indicates the unhygienic condition of the processing area. There was considerable *Vibrio* spp. observed in all samples, which represents unhygienic condition of the production pond or feed. No *Staphylococcus aureus* was detected in the samples tested. However, the presence of pathogenic bacteria like *E. coli*, *Shigella* and *Salmonella* is of special concern. This result suggested that microbiological conditions of shrimps sold in local markets are of special concern. However, most prawns or shrimps are cooked prior to consumption, these products therefore, pose minimal health risks to the consumer except for cross contamination in kitchens (18).

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