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***Research Article***

# ASSESSMENT OF BACTERIOLOGICAL QUALITY IN SELECTED COMMERCIALLY IMPORTANT PROCESSED SEA FOODS BY POLYMERASE CHAIN REACTION (PCR)

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## ABSTRACT
The quality of processed seafoods in terms of microbial and chemical parameters is determined by handling and storage conditions. The objective of this study is to assess the bacteriological quality of selected commercially important seafoods by Polymerase chain reaction (PCR). Frozen samples of seafoods which include crab meat, white shrimp, ribbon fish, grouper fillets, squid, cuttlefish and breaded fish collected from the storage facilities in Chennai, Tamilnadu, India, were screened for the bacteriological quality. The results of the study showed that the samples were contaminated with bacteria pathogens *viz*., *Staphylococcus aureus, Vibrio cholerae, Listeria monocytogenes* and *E.coli* 0157: H7 which stresses the need for hygienic processing and proper storage of seafoods in order to ensure the health and the safety of the consumers.

***Key Words***: *Seafood, Bacteriological Quality, PCR and* F*ood Hygiene*

## INTRODUCTION
Fishery products, which are of great importance for human nutrition and provide clear health benefits, can also act as a source of various food borne diseases (Darlington and Stone, 2001). One-fourth of the world's food supply and 30% of landed fish are lost through microbial activity alone (Huis *et al*., 1996). Even though the safety of food has dramatically improved overall, progress is uneven and foodborne outbreaks from microbial contamination, chemicals and toxins are still common in many countries (WHO, 2007). Among all the foodborne disease outbreaks reported globally, seafood accounts for up to 10% of all outbreaks (Huss, 2003). Therefore in order to increase the shelf life, the hygienic practices must be properly exercised to get rid of quality losses in terms of microbial and chemical parameters (Abbas *et al*., 2005). The pathogens that are normally associated with the contamination of processed seafoods are *Escherichia coli* (Candrian *et al*., 1991), *Staphylococcus aureus (*Yang *et al*., 1993), and human pathogens such as *Salmonella spp.* (Bej *et al*., 1994), *Vibrio cholera* (Koch *et al*., 1993) and *Listeria monocytogenes* (Wang *et al*., 1997). The detection of various pathogenic bacteria is primarily based on traditional microbiological culture methods and laborious, time consuming (3-5 days) identification methods (Prasad and Ambarish, 2009). Identification of the bacterial species following rapid and sensitive methods like Polymerase chain reaction (PCR) would help in early detection of microbial contamination (Malorny *et al*., 2003). PCR has been proved to be an effective tool for the detection of pathogens in food as it represents a rapid procedure for detection and specific identification of pathogenic bacteria from different food materials (Hill, 1996). Hence, the present study was carried out with an objective to assess the quality of selected commercially important processed seafoods collected from the frozen storage facilities in Chennai, Tamilnadu, India by Polymerase chain reaction (PCR) so as to evaluate their bacteriological safety.

## MATERIALS AND METHODS

### *Sample collection*

Frozen seafoods were collected from about seven frozen storage facilities in Chennai, Tamilnadu, India. The details of the types of frozen seafoods collected and used in the study and their codes are presented in Table 1. Twenty samples were collected in each type of seafood and aliquots of samples were taken aseptically and used for analyses immediately

**Table 1: Frozen Seafoods Used in the Study and Their Codes**

| S.No. | Sample | Code |
|---|---|---|
| 1. | Crab meat | CM |
| 2. | White shrimp | WS |
| 3. | Ribbonfish | RF |
| 4. | Grouper fillets | GF |
| 5. | Squid | SQ |
| 6. | Cuttlefish | CF |
| 7. | Breaded fish | BF |

### *Analysis of samples by PCR*

The aliquots of samples were first aseptically enriched in the appropriate enrichment media to allow the growth of the bacterial pathogens studied. The details of the enrichment media used for the pathogen is presented in Table 2.

**Table 2: Enrichment Media Used in the Study**

| S.No | Microorganism | Enrichment media | Supplement |
|---|---|---|---|
| 1 | *V.cholerae* | Alkaline peptone water | - |
| 2 | *S.typhimurium* | Lactose broth, Tetrathionate broth | - |
| 3 | *L.monocytogenes* | Trypticase soy broth+0.6% Yeast extract, Fraser broth base | Listeria selective supplement II |
| 4 | *S.aureus* | 0.1 % Peptone water | |
| 5 | *E.coli 0157:H7* | Modified Tryptic soy broth with novobiocin supplement | - |

**Table 3: Primers and Protocols Followed for PCR Amplification**

| Pathogens | Sequence | Reference |
|---|---|---|
| *S.aureus* | 5’ GCGATTGATGGTGATACGGTT 3’<br>5’ CAAGCCTTGACGAACTAAAGC 3’ | Wang *et al*., 1997 |
| *V.cholerae* | 5’ TGAAATAAAGCAGTCAGGTG 3’<br>5’ GGTATTCTGCACACAAATCAG 3’ | Wang *et al*., 1997. |
| *E.coli* 0157:H7 | 5’ GTAGGGAAGCGAACAGAG 3’<br>5’ AAGCTCCGTGTGCCTGAA 3’ | Wang *et al*., 1997. |
| *S.typhimurium* | 5' CGTGCTCTGGAAAACGGTGAG 3'<br>5' CGTGCTGTAATAGGAATATCTTCA 3' | Bej *et al*., 1994 |
| *L.monocytogenes* | 5’ CGGAGGTTCCGCAAAAGATG 3’<br>5’ CCTCCAGAGTGATCGATGTT 3’ | Koch *et al*., 1993 |

DNA was extracted from each of the enriched sample following the method described by Malorny *et al*. (2003). PCR was carried out with the DNA extracted from samples for various food borne bacterial pathogens of human importance. The pathogens of interest and published primers and protocols followed for PCR amplification are listed in Table 3. PCR assay was carried out in a 25 µl total volume with 22µl of 1X PCR master mix (Bangalore Genei, India), 1µl (20 pmoles) each of forward and reverse primers and 1 µl of DNA from samples. PCR amplification was performed in a PCR thermal cycler (Eppendorf, Germany) following appropriate PCR cycling conditions. The PCR products were visualized under UV after staining with ethidium bromide and the results were documented in a gel documentation system (Vilber Lourmet, France).

## RESULTS AND DISCUSSION

PCR analysis of the samples showed that they were contaminated with atleast one or more bacterial pathogens. PCR amplification of *S. aureus*, *V.* cholerae, *L. monocytogenes* and *E. coli* 0157:H7 in the samples resulted in expected product sizes of 276 bp, 777 bp, 234 bp and 361 bp respectively (Fig.1). The status of the microbial contamination by various bacterial pathogen in seafood samples as observed by PCR is shown in Fig.2. All the samples collected in the study were observed to be contaminated with *S. aureus.* None of the samples analyzed showed contamination with *S.typhimurium*. The overall percentage of bacterial contamination in seafood samples varied from 20-80% as shown in Fig.3. Crabmeat (CM ) was observed to be the most contaminated as 70% of the samples showed positivity by PCR for *S. aureus* and 70% for *V. cholerae*. Also 20% of the crabmeat sample analyzed was observed to be contaminated with *E. coli* 0157:H7. *L.monocytogenes* contamination was detected in 60% and 30% of ribbon fish (RF) and cuttlefish (CF) respectively.

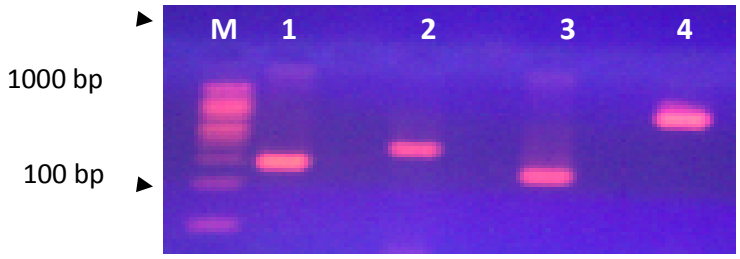


**Figure 1: PCR amplified products of bacterial pathogens from frozen seafood samples**

Lanes: M- 100bp Molecular weight marker; 1- *nuc* gene of *S. aureus*; 2- *hly* gene of *E. coli*; 3- *hemolysin* gene of *L. monocytogenes*; - *ctx* gene of *V. cholerae.*

It is well known that the spoilage of any food product is attributed to microbial growth due to improper handling, long gap between harvesting and processing and poor storage conditions (Gram and Huss, 2000). Polymerase chain reaction (PCR) is a rapid method which is highly specific and sensitive for the rapid diagnosis of food borne pathogens. PCR technique has been proven to be an effective molecular tool in detection of foodborne pathogens by various researchers (Bej *et al.*, 1994 Wang *et al.*, 1997). Various virulent gene markers have been targeted for the detection of the bacterial pathogens by PCR. PCR amplification of *nuclease* gene of *S.aureus* in the seafood samples in the present study showed that *S.aureus* contamination level was 30-80%. *S.aureus* contamination of upto 25% have been reported in marine fishes like frozen grouper and mackerels (Tayo *et al.*, 2012). Presence of *Staphylococcus aureus* is an indicator of poor handling of seafoods and its presence acts as a main causative agent of food intoxication by virtue of enterotoxins.*V. Cholerae* was detected in the present study in crab meat. Koch *et al*. (1993), Wang *et al*. (1997) have also reported *V.cholerae* contamination in crabmeat and oysters by PCR amplification of *ctx* gene of *V.cholerae*. *V.cholerae* contain as high as 70% was observed in our study. *V. cholerae* O1 outbreaks have also been reported in association with raw shellfish consumption

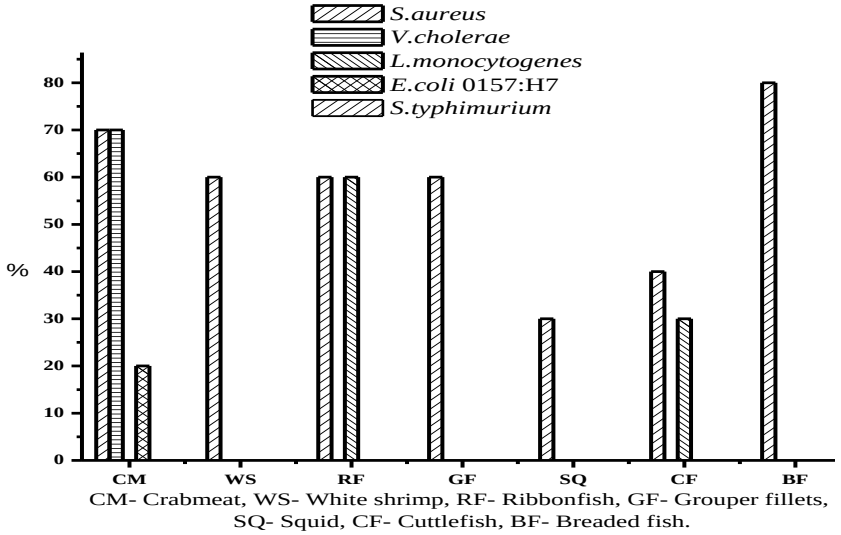


**Figure 2. Contamination of frozen seafoods with bacterial pathogens (Sample size 'n' = 20).**

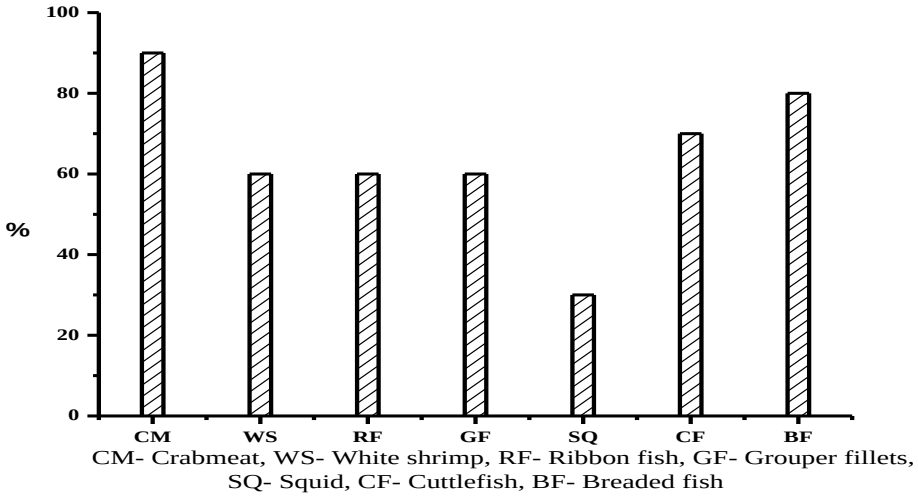


**Figure 3: Levels of Bacterial Contamination in Seafood Sample**

and raw seafood or cooked crab (Baine *et al*., 1974). All recent outbreaks in the United States have involved inadequately cooked or mishandled crustaceans such as crab and shrimp (Hackney, 1988).

Bacterial contamination with *L.monocytogenes* in seafoods like frozen Ribbonfish, cuttlefish (Wang *et al.*, 1997), shrimp and crab (Motes, 1991) have been detected by PCR. In one of the study with grouper and other seafoods, six *L. monocytogenes* isolates were recovered and rate of contamination was found to be around 10% for all the samples (Herrera *et al.*, 2006), whereas in our study the level of contamination were 60% in ribbonfish and 30% in cuttlefish. Therefore, the presence of L. *monocytogenes* on displayed raw marine fish from clean waters most likely represents handling and processing contamination. *E. coli* is a classic example of enteric bacteria causing gastroenteritis. *E. coli* 0157: H7 is not very much associated with the seafoods but it gain access to the sea foods through cross contamination. *HlyA* gene of *E.coli* was amplified by PCR for the detection of *E. coli* 0157:H7 in the present study. Detection of *E.coli* 0157: H7 by amplification of this gene has already been reported in seafoods such as crabmeat, shrimp, crayfish, salmon, oysters (Wang *et al.*, 1997). As crabmeat alone showed positivity for *E. coli* 0157:H7 in our study and the contamination might be due to the use of faecal contaminated water during processing.

Fish and shellfish appear to be passive carriers of salmonella, since this species is mainly present in the gastrointestinal tract of animals and birds. The consumption of food contaminated with salmonella leads to salmonellosis followed by various enteric diseases. In the present study *S. typhimurium* was not detected in any of the samples analyzed. The absence of this bacterium was confirmed by the PCR amplification of *him*A gene. The similar result has been observed in the study involving seafoods such as grouper, sole, swordfish, conger (Herrera *et al.*, 2006). The result of this study highlights the contamination of the frozen seafoods by bacterial pathogens and necessitates the need to adopt stringent quality control measures during handling and storage of seafoods.

## CONCLUSION

The objective of this study was to access the bacteriological quality in the frozen seafood samples collected from the frozen storage facilities in Chennai, Tamilnadu, India by Polymerase chain reaction (PCR). The bacterial pathogens detected in the seafood samples include *Staphylococcus aureus, Vibrio cholerae, Listeria monocytogenes* and *E.coli* 0157: H7 which reflects the unhygienic processing and storage practices. The results also stress the need to adopt the stringent practices in each and every step of processing and storage so as to meet the bacteriological quality standards of frozen seafoods.

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