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Incidence of Food borne Pathogens in Seafood Sourced from Neendakara and Thangassery Harbours in Kollam, Kerala, India

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ABSTRACT

Seafood is a major foreign exchange earner and the microbiological quality of exported fish and shellfish is crucial for their safety and acceptance in international trade. However, seafood that is sold for local consumption in India undergoes very limited safety or quality checks. If fish are not harvested, processed and handled hygienically, they can harbour pathogenic microorganisms that can cause foodborne infections and intoxications and become a major health concern. The present work was carried out to determine the total viable bacterial count and to evaluate the presence of bacterial pathogens, such as *Escherichia coli, Salmonella* and *Vibrio cholerae,* on the surface of fish and prawns collected from Neendakara and Thangassery harbours in Kollam district during the monsoon season. The total viable aerobic plate count in the seafood ranged from 2.74-3.08 log CFU cm^{-2}. The fish collected from both the harbours as well as the prawns collected from Thangassery harbour were contaminated with *E. coli*. The samples of prawns collected from Neendakara were found to contain *V. cholerae*, a pathogen that should be absent in 25 g of seafood as per the Food and Safety Standards Authority of India (FSSAI). *Salmonella* was not detected in any of the samples. Crushed ice collected from the nearby ice plants did not show any evidence of the tested pathogens, suggesting that the seafood contamination may have come from other sources such as contact with surfaces, handlers or polluted water. Therefore, proper monitoring and control measures need to be implemented by relevant authorities to ensure the quality and safety of domestic seafood and prevent seafood-associated infections.

Key words : *Seafood, Contamination, Pathogens, E. coli, V. cholerae, Kerala, Monitoring*

Introduction

Globally, food-borne diseases are not only a significant public health problem but also an economic problem due to the loss of productivity, wages, and other expenses. Food-borne outbreaks in India have increased from 37 during 1980-2010 (Sudershan *et al.*, 2011) to 2688 during 2009-2018 (Bisht *et al*., 2021). The states of West Bengal, Karnataka and Gujarat had the highest average outbreaks during 2009-2018 (Bisht *et al*., 2021). At the current state of food safety standards, the number of food-borne diseases in India will escalate to 150–177 million cases annually by 2030 (Kristkova *et al*., 2017). A 2009-2018 study

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published by the US Centers for Disease Control and Prevention (CDC) showed that fish was the most common type of food implicated in outbreaks involving a single food category (CDC, 2018). Given the significant increase in fish consumption over the past 40 years, it is important to ensure that fish is of good quality and free from contamination for the sake of food safety. Seafood can be contaminated by pathogens at any point along the supply chain, from harvesting to the table. *Escherichia coli, Salmonella, Vibrio, Listeria, Shigella, Staphylococcus* and *Clostridium* are the most prevalent pathogens implicated in seafood poisoning (Afreen and Ucak, 2021). The bacterial population present in fish is a reflection of those in their habitat (Novoslavskij *et al.*, 2016). Also, the quality of the water and the crushed ice used for cleaning and chilling the fish affect the microbial quality of fish. According to FSSAI, 2011, *E. coli* is permitted up to 20 CFU g^{-1} while, *Salmonella* and *Vibrio cholerae* must be absent in 25g of the seafood sample. These standards are followed very strictly for exported food, however, such quality measures are not ensured in the case of domestically sold seafood. Therefore, to assess the microbiological quality of the local seafood, the total bacterial count and the presence of pathogens namely *Escherichia coli, Salmonella* and *Vibrio* on the surface of the seafood samples and in crushed ice sourced from Neendakara and Thangassery harbours in Kollam, Kerala, India was determined.

Materials and Methods

Collection of seafood samples and ice

The captured fish (*Opisthopterus* sp.) and prawns (*Fenneropenaeus* sp.) were collected separately in sterile polyethylene bags from both Neendakara and Thangassery harbours in Kollam. The samples were collected in the early morning during May-June 2021 and brought in ice to the laboratory and processed within 3 hours. Ice was also collected from the nearby ice plants and processed similarly.

Enumeration of Total viable aerobic count (TVAC) of Fish, Prawn and Ice

The samples for analysis were taken by aseptically swabbing 25 cm^2 area of the surface of fish or shell-on prawns with separate swabs premoistened with 0.1% buffered peptone water (pH 7.0). The swabs were then put back in 100 ml of the peptone water diluent to obtain a 10^{-2} dilution. 1 ml of the 10^{-2} diluent was transferred to duplicate Petri dishes and about 18 ml of molten plate count agar (PCA) was poured into it, mixed and allowed to solidify. The plates were incubated in an inverted position at 36±1°C for 48 hrs. 1 ml of the ice sample was serially diluted in buffered peptone water up to 10^{-2} and plated 1 ml of the original sample and the different dilutions in duplicate plates, poured 18 ml of molten plate count agar (PCA), mixed and allowed to solidify. After incubation in an inverted position at 36±1°C for 24-48 hrs, the plates having colonies in the range of 25-250 were used for counting. Total viable aerobic count per cm^2 was determined by multiplying the average number of colonies for each dilution by the corresponding dilution factor and expressed as log colony forming units (CFU) per cm^2 of the seafood samples or log CFU per ml for the ice samples.

Detection of *Escherichia coli, Salmonella* and *Vibrio*

Standard methods for the detection of *E. coli, Salmonella* and *Vibrio cholerae* [IS 5887-1: 1995, IS 15187: 2002 IS15188: 2002 and IS 5887-5 (1976), reaffirmed 2005] were employed. Briefly, 25 g of the seafood sample or ice was homogenised with 225ml of 0.1% buffered peptone water (pH 7.0) to get 10^{-1} dilution. For the growth of *E. coli*, 10^{-1} dilutions were inoculated into MacConkey broth with Durham's tubes and observed for growth and gas formation. Tubes showing gas formation were streaked on Tergitol-7 agar, MacConkey agar and EMB agar and incubated at 36±1°C for 24-48 hrs. The colonies showing the characteristic presence of *E. coli* were then transferred into suitable media for IMViC tests for confirmation. For *Salmonella*, 25g of the samples were shaken with 250 ml of buffered peptone water (pre-enrichment), filtered and the membrane filter incubated in buffered peptone water overnight for pre-enrichment. To enhance the likelihood of obtaining all *Salmonella* strains, a secondary pre-enrichment was done in which the pre-enrichment culture was inoculated into Kauffman tetrathionate novobiocin broth (MKTNB) and Rappaport-Vassiliadis soya peptone broth (RVS) and both incubated at 36±1°C for 18-24 hours. Loopfuls from each of MKTNB and RVS were streaked separately onto brilliant green agar (BGA) and xylose lysine deoxycholate agar (XLD) and incubated at 36±1°C for 20-24 hours. For confirmation of *Salmonella*, Kligler Iron agar, Urease test and polyvalent serum were used. Enrichment

for *Vibrio* was done by transferring 25g of the sample to 225 ml of alkaline peptone water and incubated without shaking at 36±1°C. After 18 hours without shaking, a loopful of inoculum was taken from the surface pellicle and streaked onto TCBS agar and Bile salt agar and incubated at 36±1°C for 18-24 hrs. Further, polyvalent cholera typing serum, Triple Sugar Iron agar test, Catalase test, growth in Tryptone water and Gram staining were done.

Results

As shown in Table 1, the total viable aerobic counts of bacteria on the fish skin and prawn surface from the Neendakara and Thangassery harbours ranged from 2.74-3.08 log CFU cm^{-2}. The ice collected from ice plants near the harbours had an average TVAC of 1.1 log CFU g^{-1} which was within the limit of < 1.3 log CFU ml^{-1} specified by ICMSF (1986).

Fish sourced from Thangassery and Neendakara harbours were *E. coli* positive and isolates gave the characteristic smooth, non- mucoid yellow colonies surrounded by yellow zone on T7 agar, pink coloured colonies in MacConkey agar and blue-black colonies with greenish metallic sheen in Eosin Methylene Blue (EMB) agar respectively in the preliminary tests. The bacterial isolates suspected to be *E. coli* on the different media were subjected to biochemical tests for confirmation. The samples were Indole positive, MR and gas production positive, VP negative and Citrate negative, indicating the presence of *E. coli*. *E. coli* isolates were urease and H_2S production negative during their biochemical test.

Prawn samples obtained from Neendakara harbour showed the presence of smooth yellow flattened colonies with opaque centre and translucent peripheries on Thiosulphate citrate Bile salts sucrose agar (TCBS) medium which is selective for cholera *Vibrio*. The suspected *Vibrio* colonies which tested positive for the preliminary slide agglutination test were subjected to Triple Sugar Iron Agar (TSI), oxidase and catalase biochemical tests. In TSI, no H_2S was produced as indicated by the absence of a black precipitate. The catalase test gave a positive reaction. Together, these results indicated the presence of *V. cholerae* in the prawn samples from Neendakara. However, *E. coli* and *Salmonella spp* were not detected in these samples.

The prawn samples from Thangassery harbour on the other hand, showed smooth, pink and lactose fermenting colonies on MacConkey agar, blue black colonies with greenish metallic sheen on EMB agar and smooth, non- mucoid yellow colonies with rust brown centre on T7 agar. The biochemical analyses of these colonies showed + + - - result for the Indole-Methyl Red-Voges-Proskaeur-Citrate, (IMViC) Test respectively. From the results obtained, it is clear that the prawn sample from Thangassery landing site was contaminated with *E. coli* suggesting the occurrence of faecal contamination. However, these prawn samples tested negative for S*almonella spp*. and *Vibrio* spp.

No typical colonies of *E. coli, V. cholerae* or *Salmonella* were observed from ice on any of the selective media indicating that the ice samples were free from these pathogenic bacteria. The results of these analy-

Table 1. Total viable aerobic count (TVAC) on live fish skin, prawn surface and crushed ice sourced from Neendakara and Thangassery harbours in Kollam, Kerala, India. Results are expressed as mean ± standard deviation.

	Neendakara Harbour	Thangassery harbour
Fish skin (log CFU cm^{-2})	2.87 ± 1.5	2.74 ± 1.1
Prawn surface (log CFU cm^{-2})	3.08 ± 2.0	2.97 ± 1.9
Crushed ice (log CFU ml^{-1})	0.85 ± 0.5	1.24 ± 0.8

Table 2. Detection of *Escherichia coli, Salmonella* spp., and *Vibrio cholerae* contaminationin fish, prawn and crushed ice samples collected from Neendakara and Thangassery harbours in Kollam, Kerala, India.

Source	Sample	*Escherichia coli*	*Salmonella*spp	*Vibrio cholerae*
Neendakara Harbour	Fish	+	-	-
	Prawn	-	-	+
	Crushed ice	-	-	-
Thangassery Harbour	Fish	+	-	-
	Prawn	+	-	-
	Crushed ice	-	-	-

ses are summarized in Table 2.

Discussion

The safety of fish and fish products is significantly influenced by microorganisms. The bacterial count on the surface of fish and prawns can vary depending on a number of factors, including the type of fish or prawn, the habitat in which it lives and its health status. In this study, we observed an average total aerobic count of about 2.8 and 3.0 log CFU cm^{-2} on the surface of the fish and prawns respectively, collected from the two Kollam harbours. The prawn samples were found to have a higher bacterial load compared to the fish, which is expected as they are bottom dwelling organisms and are contaminated with mud that is often trawled up with them (Adams and Moss, 2000). Lakshmi *et al.,* 2021 observed bacterial counts in the range of 3.84- 4.88 log CFU g^{-1} in the skin of Mugil fish collected from different sites in Ashtamudi Lake in Kollam. In this study, the investigated samples from the Arabian Sea had a comparatively higher bacterial load possibly because of the difference in the source and type of fish and the sampling methods employed. A study of the bacterial count on the skin surface of *Etroplus suratensis* collected from upstream and downstream regions of the Mogral river in Kasargod, Kerala was 4.41 and 4.92 log CFU g^{-1} respectively (Megha and Harikumar, 2016). Jahan *et al* (2019) showed that the bacterial count on the surface of the fish, which varied with the species and the source, ranged from 3.5 to 5.17 log CFU g^{-1}.

In several microbial analyses of fish conducted in different parts of India, high bacterial counts and pathogens such as *E. coli, Vibrio sp., Salmonella, Enterobacter sp., Shigella etc* were recorded on the skin, gills and other parts of the fish body (Sujatha *et al.,* 2011; Velappan and Munuswamy, 2016; Dutta *et al.,* 2015). The quality of seafoods depends not only on the quality of the waters from where the fish are captured but also on the cleanliness of the contact surfaces, the fish handlers, the landing centres and the ice which is used for storage. According to Pal *et al*, 2016, crushed ice was usually a major source of contamination of seafoods. In this study, the bacterial load in the crushed ice sourced from the nearby ice plants was very low (<10 CFU g^{-1}) and none of the tested pathogens were detected. This may be due to the improved facilities and good hygienic practices of the ice plant authorities. However, the crushed ice, could become a source of contamination after repeated reuse and coming in contact with different seafoods, surfaces and handlers.

In this study, *E. coli* was detected in the fish samples collected from both the harbours and in the prawn sample from the Thangassery harbour. *E. coli* contamination of seafood is quite common in several parts of India (Anthony *et al.,* 2002; Thampuran *et al*., 2005; Sehgal *et al.,* 2008; Jeyasanta *et al*., 2012, Singh *et al*., 2016; Dutta and Sengupta, 2016; Singh *et al.,* 2017; Prakasan *et al.,* 2018; Singh *et al.,* 2020). In the present study, the *E. coli* contamination could have occurred during postharvest handling rather than from the fishing waters as *E. coli* was undetected in one of the prawn samples. Although we did not test the antibiotic resistance patterns of the *E. coli* isolates, the occurrence of multidrug resistant *E. coli* as well as pathogenic *E. coli* O157 isolated from seafood in India (Sehgal *et al*., 2008; Singh *et al*., 2016, Singh *et al.,* 2020) is particularly concerning as they can endanger the health of consumers.

According to FSSAI, 2011, pathogens such as *Salmonella* and *Vibrio cholerae* must be absent in 25g of fresh or frozen seafood samples. Although we did not detect any *Salmonella* in any of the seafood samples, *Vibrio cholerae* was present in the prawn samples from Neendakara harbour. *V. cholerae* is known to be abundant in aquatic habitats and its presence in seafood is inevitable (Raymond and Ramachandran, 2019). Several studies in India have reported the occurrence of *V. cholerae* in wild caught and farmed seafood (Saravanan *et al*., 2007; Kakatkar*et al*., 2010; Sujatha *et al*., 2011; Kumar and Lalitha, 2013; Madhusudana and Surendran, 2013: Sudha *et al*., 2014; Joseph *et al*., 2015; Dutta *et al*., 2015).

Conclusion

Since seafood is cooked properly in Indian households, the chances of foodborne infections due to consumption of contaminated seafood are low. However, cross contamination of other foods or surfaces due to the use of the same cutting and processing utensils can present a health risk resulting in abdominal cramps, diarrhoea, nausea, vomiting, etc. Hence, to reduce the risk of foodborne infections from fish and other seafood, it is important to follow safe food handling and preparation practices. In addition, regular testing of locally sold seafood samples and ensuring clean and hygienic conditions

during harvesting, landing, transportation and processing of seafood is essential to prevent any future outbreaks.

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Conflict of interest

None.

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