Original Research Article

Microbial Quality Evaluation of Seafood Samples from the Vishakhapatnam Coast, Andhra Pradesh

Abstract:

Consumption of seafood has increased, resulting in the production and productivity of aquaculture in the past few years. Since, seafood is crucial in human nutrition, providing essential nutrients and proteins. However, their perishable nature and vulnerability to microbial contamination make them prone to spoilage and foodborne illnesses. Therefore, the microbiological analysis of fish samples is paramount to ensure their quality and safety for consumption. In this study, presents an overview of the microbiology of fish samples, encompassing various aspects such as microbial load assessment, and pathogen detection. Isolation and identification of pathogenic bacteria *viz.,Total plate count, Escherichia coli,*Total coliforms, *Vibrio* spp, *Staphylococcus aureus*,and*Salmonella* spp. These pathogens pose severe health risks to consumers and highlight the necessity of effective monitoring and control measures throughout the seafood supply chain.Proper handling, storage, and processing practices minimise microbial contamination and preserve fish quality. Implementing Hazard Analysis Critical Control Point (HACCP) plans and Good Manufacturing Practices (GMP) helps ensure the safety of fish products and prevent outbreaks of foodborne illnesses.

Keywords: Microbiological quality, fish, shellfish, seafood,

Introduction

Fish and shellfish play a significant role in human health, having many beneficial parts in reducing heart diseases, brain function, bone formation, reproduction, etc.. Since the olden days, fish and shellfish have been a speciality in traditional cooking, especially in Andhra Pradesh and the whole of India (1). Andhra Pradesh has a vast coastline stretching over 974 kilometres along the Bay of Bengal and is located on the eastern coast of the Indian subcontinent. This abundant coastline and its extensive network of rivers, lakes, and ponds provide the ideal environment for a rich

diversity of fish and shellfish species to thrive (2). The coastal region of Vishakhapatnam, located in Andhra Pradesh, India, is known for its rich marine biodiversity and abundant seafood resources. The coastal waters of Vishakhapatnam provide a favourable environment for the growth and sustenance of various seafood species, making it a significant hub for fishing and aquaculture activities. With the growing demand for seafood products both domestically and internationally, ensuring the quality and safety of these products has become paramount.

The coastal regions of Andhra Pradesh are renowned for their vibrant fishing communities, whose livelihoods are intricately tied to the abundant marine resources of the area. The traditional fishing villages, with their colourful boats and busy fish markets, offer a glimpse into the coastal way of life, where fish and shellfish have been a staple food for generations.The fishing industry is crucial in providing employment opportunities to thousands of people, directly and indirectly, from fishermen to fish vendors and processors.Quality evaluation of seafood samples from the Vishakhapatnam coast plays a crucial role in maintaining consumer confidence, safeguarding public health, and supporting the sustainable development of the local fishing industry. Rigorous evaluation protocols are employed to assess the freshness, microbiological safety, chemical composition, and overall quality of the seafood harvested from this region.

Microbes are ubiquitous in nature and pathogenic microorganisms like *E.coli, Vibrio cholera, Salmonella typhi, Salmonella paratyphi, Staphylococcus aureus, Listeria monocytogens* are some of the pathogens of public health interest. These pathogens cause severe pose to human health, causing diseases and sometimes leading to death (3). Fresh seafood is a valuable source of essential nutrients and proteins; however, it can also harbour various microorganisms that may pose a risk to human health. Microbiological analysis of seafood plays a crucial role in ensuring the safety and quality of these products. Thestudy aims to investigate the microbial quality evaluation of seafood samples from the fishing harbour of Vishakhapatnam, Andhra Pradesh.

Materials and Methods

Sample collection

The samples obtained comprised fish, shellfish and crustaceans freshly caught and were brought to the landing centres in the fishing harbour of Vishakhapatnam. The samples were collected in sterile polythene bags and appropriately labelled and brought to the laboratory in an insulated container having the ice in the ratio of 1:1 under controlled conditions.

Sample Preparation

The seafood samples were gently washed with sterile distilled water to remove surface debris and excess contaminants. Aseptically, the fish and shellfish samples were cut and blended the sample into smaller, homogeneous portions using sterile pestle and motor for further dilutions using buffered peptone water to obtain a suitable range of colony counts for accurate analysis(4).

Microbiological Analysis:

The following microbiological analyses were conducted on the seafood samples:

a. Total Plate Count (TPC):

The total plate count was done using the spread plate method onto the Plate count agar. The samples were evenly spread over the agar surface using a sterile glass spreader. The plates were incubated at 37^{o}C temperature for 24 to 48 hours. The colonies on the plates ranging were counted and reported as colony-forming units per gram (CFU/g) (4).

b. Total Coliform Count:

The coliform count was determined using the most probable number (MPN) method. Serial dilutions of the samples were prepared and inoculated in the test tubes containing about 9mL Lauryl Tryptose Broth (LTB) with an inverted Durham's tube. Three test tubes were inoculated with 1mL from 10^{-1} dilution, another three inoculated from 10−2 dilution, and the remaining three inoculated from from10^{-3}

dilution. The inoculated tubes were incubated at 37°C for 24-48 hours. Test tubes showing positive results (gas production in Durham's tube) were counted and recorded as presumptive positive for coliforms. MPN values were determined using the MPN table(5) (Feng et al., 2002).

c. *E. Coli* Analysis:

MacConkey agar was used for selective enrichment of *E.coli* detection, and the samples were evenly spread. The inoculated plates were incubated at 37°C for 24 hours. The suspected *E.coli* colonies, based on their characteristic appearance on the selective media, were identified using the battery of biochemical tests(5) (Feng et al., 2002).

d. *Staphylococcus aureus*

Baird Parker Agar was used for the enrichment of *S.aureus* isolation and confirmation through catalase and coagulase test(6) (Bennett and Lancette 2016). The sample inoculums were evenly spread, and the triplicate plates were incubated at 37°C for 24 hours. The colonies were confirmed using the coagulase and catalase tests.

e. Vibrio Analysis:

About 25 g samples were blended with 225mL sterilized Alkaline Peptone Water (APW) and incubated at 37°C for 16– 18 hours. Then 1 loopful inoculum from the APW culture was streaked on the preincubated thiosulfate citrate bile salts sucrose agar (TCBS) agar plate and incubated at 37∘ for 24 hours(7). Typical colonies of *V. cholerae* on TCBS agar are large, yellow, and smooth.

f. Salmonella Analysis:

About 25 g samples were dissolved in about 225mL of sterilized buffered peptone water (BPW), blended, and incubated at 37∘C for 16–20 hours. About 10mL from the set BPW culture was selectively enriched into the 100mL sterilized Selenite Cystine Broth and incubated again at 37∘C for 24–48 hours. After incubation, one loopful inoculum fromthe selective enrichment culture was streaked onto the preincubated

BSA and XLD agar plate. Typical *Salmonella* spp. produce pink colonies with or without black centers on XLD agar and brown, grey, or black colonies on BSA agar(8).

Results and discussion

The microbiological analysis was conducted on the samples collected from fresh seafood caught obtained from the Vishakhapatnam coast of Andhra Pradesh and samples received from the market. The samples were assessed for their Total Viable Count (TVC), presence of coliforms, *Escherichia coli*, and *Salmonella* spp. These parameters are indicative of the microbiological quality and safety of the seafood. Seafood secured an important place in the food component and very essential for the improvement of human health. Pathogenic microbes cause serious threats to human health. Microbial quality evaluation of seafood is essential for safe food. The fresh samples have the Total Plate Count (TPC) ranging from 4.9×10^3 CFU/g to 5.8×10^6 CFU/g. The shrimp samples range from 5.2×10^4 CFU/gto 2.7×10^6 CFU/g, and the crab samples range from 6.5×10^4 CFU/g to 4.6×10^7 CFU/g. The counts obtained suggest that the seafood samples have a moderate level of microbial contamination.

The coliforms of fish samples are<10 MPN/g; shrimp samples<10 MPN/g; and crab samples<10 MPN/g. The coliform counts in all samples were below 10 MPN/g, indicating a low level of faecal contamination. This is an encouraging finding, as coliforms are used as indicators of potential pathogens. The absence of *E. coli* in all samples suggests no faecal contamination and the seafood is free from this specific indicator bacterium. But the coliform counts revealed the presence of faecal contamination in some seafood samples. The MPN values ranged from 1.2 MPN/g to 6.7 MPN/g, with higher counts observed in shellfish samples than fish and crustaceans. However, *E.coli*; *Vibrio* spp. and *Salmonella* spp. were absent in the fish, shrimp and crab samples. However, *E.coli* is present in the samples brought from the market. The absence of *Salmonella* spp. in all samples is a positive result, as *Salmonella* is a common pathogen associated with seafood-borne illnesses. The absence of *Salmonella* spp. is encouraging, suggesting that proper food safety protocols are being followed. *Staphylococcus aureus* was found positive in the samples brought from the market place and absent in the samples of seafood catch.

This might be due to the contamination in the equipment's, handling etc. *Vibrio cholerae* is responsible for the third-highest number of shellfish-related illnesses, after noncholera *Vibrio* spp. and Norwalk viruses (9).In contrast to *Vibrio* spp., the incidence of *Salmonella* infections due to seafood consumption is still low compared with salmonellosis associated with other foods. However, the detection of *Salmonella* spp. in seafood cannot be skipped as it is responsible for most of foodborne diseases or gastroenteritis characterized by diarrhoea, abdominal cramp, vomiting, nausea, and fever. According to the Centers for Disease Control and Prevention, Salmonella is the leading cause of bacterial foodborne illness-causing approximately 1.4 million nontyphoidal illnesses, 15,000 hospitalizations, and 400 deaths in the USA annually(10, 11).

Conclusion

The microbiological analysis suggests that the fresh seafood catch from the Vishakhapatnam coast of Andhra Pradesh has moderate microbial contamination, with the absence of *Salmonella* sp., *Vibrio* sp. However, *E.coli* and *S.aureus* was detected in the samples brought from the market. This indicates the importance of handling and store of seafood. Hence, properly maintaining its freshness and minimisingpotential bacterial growth during transportation and storage. Regular monitoring of the microbiological quality of seafood is essential to ensure consumer safety and prevent foodborne illnesses.Implementing stringent Hazard Analysis Critical Control Point (HACCP) plans and Good Manufacturing Practices (GMP) helps ensure the safety of fish products and prevent outbreaks of foodborne illnesses.

References

1. Dhanapal, K., Devivaraprasad Reddy, A. and Reddy, G.V.S. 2011. Beneficial effects of fish oil and its applications in human health. *International Journal of Medicine, Biological Frontiers,* **17**(12): 1-12.
2. Anon 2023. Department of fisheries, Govt of India retrieved the information from https://dof.gov.in/documents/state-fisheries-profile/andhra-pradesh accessed on 18.08.2023.
3. Huss, H.H; Ababouch, L; Gram, L. 2003. Assessment and management of seafood safety and quality. *FAO Fisheries Technical Paper. No.* 444. Rome, FAO. 230p.

4. Jha, A.K. 2020. Enumeration of Microorganisms by Plating Technique. In: Microbial Quality Analysis of Fish and Fishery Products. Edited by Joseph, T.C., Jha, A.K., Renuka, V., Remya, S. and Anupama, T. K. Veraval Research Centre of ICAR - Central Institute of Fisheries Technology, Gujarat, India. 42-51.
5. Feng, P., Weagant, S.D., Grant, M. A. and Burkhardt, W. (2002). Bacteriological Analytical Manual, Chapter 4, USFDA.
6. Bennett, R. W. and Lancette, G. A. 2016. Chapter 12. Staphylococcus aureus. Bacteriological Analytical Manual (BAM). USFDA (Food and Drug Administration).
7. Kaysner, C.A and DePaola, Jr. A (2004) Bacteriological Analytical Manual, Chapter 9, USFDA.
8. Andrews,W.H., Wang,H., Jacobson,A. and Hammack, T. 2019. Chapter 5. Salmonella. Bacteriological Analytical Manual (BAM). USFDA (Food and Drug Administration).
9. Wittman R. J. and G. J. Flick, “Microbial contamination of shellfish: prevalence, risk to human health, and control strategies,” Annual Review of Public Health, vol. 16, pp. 123–140, 1995.
10. Centers for Disease Control and Prevention (CDC), “Preliminary foodnet data on the incidence of infection with pathogens transmitted commonly through food,” Morbidity and Mortality Weekly Report, vol. 59, no. 14, pp. 418–422, 2010, http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5914a2.html.
11. Sohana Al S. and Karim, M.K.. 2016. Microbiological Quality Assessment of Frozen Fish and Fish Processing Materials from Bangladesh. International Journal of Food Science. 1-6.