



***Vibrio alginolyticus* Occurrence in Various Fish and Shellfish Samples: A Comparative Analysis**

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ABSTRACT: *Vibrio alginolyticus* is a marine bacterium that can cause severe foodborne illnesses. The study aimed to assess the prevalence of *V. alginolyticus* in various fish and shellfish species collected from Karwar Fishing Port, Baithkol, Uttara Kannada. A total of n=120 fish and shell fish samples belong to different families and tidal zones in ocean and estuarine environment were analyzed to isolate *V. alginolyticus* using standard microbiological techniques. The results revealed a significant presence of *V. alginolyticus* in the seafood samples. *Meretrix meretrix* exhibited the highest contamination rate at 66.6%, followed by *Mugil cephalus* with 58.33%. Whereas, *Lactarius lactarius* showed the least contamination rate at 16.6%. Other species also showed varying levels of contamination. The contamination of the pathogens were common in the benthic organisms when compared to pelagic. The findings underscore the potential health risks associated with consuming raw or undercooked seafood. To mitigate these risks, stringent food safety measures, including proper handling, storage, and cooking practices, are crucial. Further research is necessary to identify factors contributing to *V. alginolyticus* contamination in seafood and develop effective prevention strategies.

KEYWORDS: Contamination, Fish, Karwar, Shellfish, Occurance, *Vibrio alginolyticus*.

INTRODUCTION

Vibrio alginolyticus, a marine bacterium, poses a significant public health concern due to its ability to cause severe foodborne illnesses. This bacterium is widely distributed in coastal waters and has been frequently implicated in outbreaks in diseases associated with the consumption of contaminated seafood. The presence of *V. alginolyticus* in fish and shellfish can lead to severe gastrointestinal symptoms, including diarrhea, vomiting, and abdominal pain [1].

The prevalence of *V. alginolyticus* in seafood varies depending on various factors, such as geographical location, water temperature, and seasonality [2]. Studies have shown that warmer water temperatures and increased salinity can promote the growth and survival of this bacterium. Additionally, the presence of *V. alginolyticus* in seafood can be influenced by factors like post-harvest handling, storage conditions, and cooking practices.

Despite the potential health risks associated with *V. alginolyticus* contamination, the consumption of fish and shellfish remains a popular choice worldwide. Therefore, it is crucial to understand the prevalence of this bacterium in seafood and to implement effective food safety measures to prevent its transmission.

Vibrio alginolyticus is a Gram-negative bacterium commonly found in marine environments and associated with various seafood products. The prevalence of *V. alginolyticus* in seafood has been a significant concern due to its potential to cause foodborne illnesses. The present study was aimed to screen the *Vibrio alginolyticus* from the commercially important fish and shellfish from the Karwar Coastal waters, Uttara Kannada Karnataka.

MATERIALS AND METHODS

Sample Collection

A total of 120 fish and shellfish samples were collected from Karwar Fishing Port, Baithkol, Uttara Kannada, India. Samples belonging families and tidal zones, including both oceanic and estuarine environments. Total of 120 fish and shell fish samples belonging to 7 families and 10 species like *Rastrelliger kanagurta*, *Sardinella longiceps*, *Sardinella fimbriata*, *Escualosa thoracata*, *Lactarius lactarius*, *Mugil cephalus*, *Anodontostoma chacunda*, *Megalaspis cordyla*, *Otolithes ruber* and *Meretrix meretrix* were collected to study the prevalence of *Vibrio alignolyticus* in the these fishes (Fig.1-10).



Figure1: *Rastrelliger kanagurta*



Figure 2: *Sardinella fimbriata*



Figure 3: *Otolithes ruber*



Figure 4: *Lactarius lactarius*



Figure 5 : *Anodontostoma chacunda*



Figure 6 : *Mugil cephalus*



Figure 7 : *Escualosa thoracata*



Figure 8 : *Sardinella longiceps*



Figure 9 : *Meretrix meretrix*



Figure 10 : *Megalaspis cordyla*

Sample Processing

The fish and shell fish samples were collected in sterilized bags and immediately transported to the laboratory in cold cold condition. The fish and shell fish were dissected to obtain the muscle tissue. The tissues were homogenised in sterile saline using pestal and mortar. The samples were inoculated in the peptone water and incubated at 34⁰C for 24hrs for the enrichment of the organisms.

Screening of *Vibrio alginolyticus*

The incubated enriched culture was inoculated on the Thiosulfate Citrate Bile Sucrose (TCBS) agar plates and incubated 34⁰C for 24hrs. The suspected yellow and yellowish green colonies were further purified and the biochemical test (Catalase test, Oxidase test, Salt tolerance test(0 10%), Indole test, Citrate Utility test, VP-MR test, Nitrate test, VP-MR test, Nitrate test, Urease test, Carbohydrate fermentation test) identified using Noguera and Blanch 2008 identification keys.

Confirmation of *Vibrio alginolyticus*

The molecular identification was done using 16S rDNA sequencing was done. The bacterial DNA was isolated and evaluated on 1.0% Agarose Gel, a single band of high-molecular weight DNA was observed. Fragment of 16S rDNA gene was amplified by 27F and 1492R primers. A single discrete PCR amplicon band of 1500 bp was observed when resolved on Agarose gel. The Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with forward primer and reverse primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of 16S rDNA gene was generated from forward and reverse sequence data using aligner software. The 16S rDNA gene sequence was used to carry out BLAST with the database of NCBI genbank database.

RESULTS

The present study was carried out to study the occurrence of *Vibrio alginolyticus* in the commercially important edible fish and shell fishes. 120 fish and shellfishes belonging to 7 families, 10 different species. Out of 120 fishes and shellfish samples were tested, 45 samples were positive for the *Vibrio species*. The biochemical and molecular identification revealed the presence of *Vibrio alginolyticus* in the sea food samples (Fig.11-15).

The results revealed a significant presence of *Vibrio alginolyticus* in the seafood samples. Among the species analyzed, *Meretrix meretrix* exhibited the highest contamination rate at 66.6%, followed by *Mugil cephalus* with 58.33%. In contrast, *Lactarius lactarius* showed the least contamination rate at 16.6% (Fig.16). It was observed that the contamination of *V. alginolyticus* was more prevalent in benthic organisms compared to pelagic organisms. The similar observation was observed in *Meretrix meretrix* of Kali and Aghanashini Estuary by Revankar, S. K *et.al.*,(2023, 2024). This suggests that bottom-dwelling species may be more susceptible to contamination by this pathogen.

The findings of present study underscore the potential health risks associated with consuming raw or undercooked seafood. The presence of *V. alginolyticus* in these samples highlights the importance of implementing stringent food safety measures to mitigate these risks.



Figure 11 : *Vibrio species* on TCBS

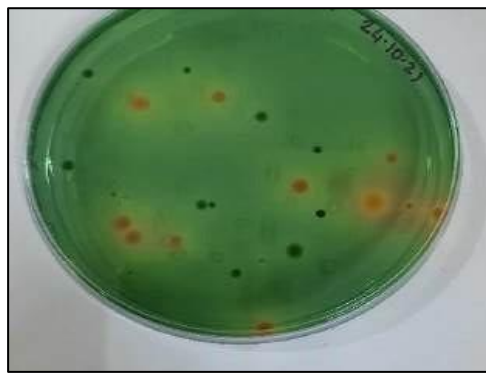


Figure 12 : *Vibrio species* in TCBS



Figure 13 : Purified *Vibrio alginolyticus*

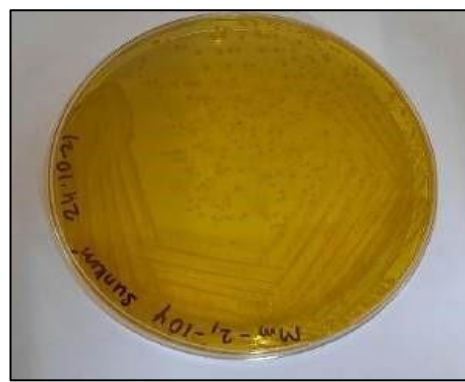


Figure 14 : Purified *Vibrio alginolyticus* on TCBS

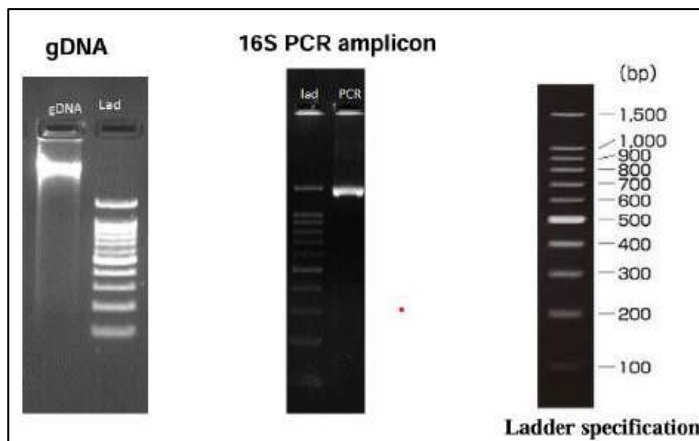
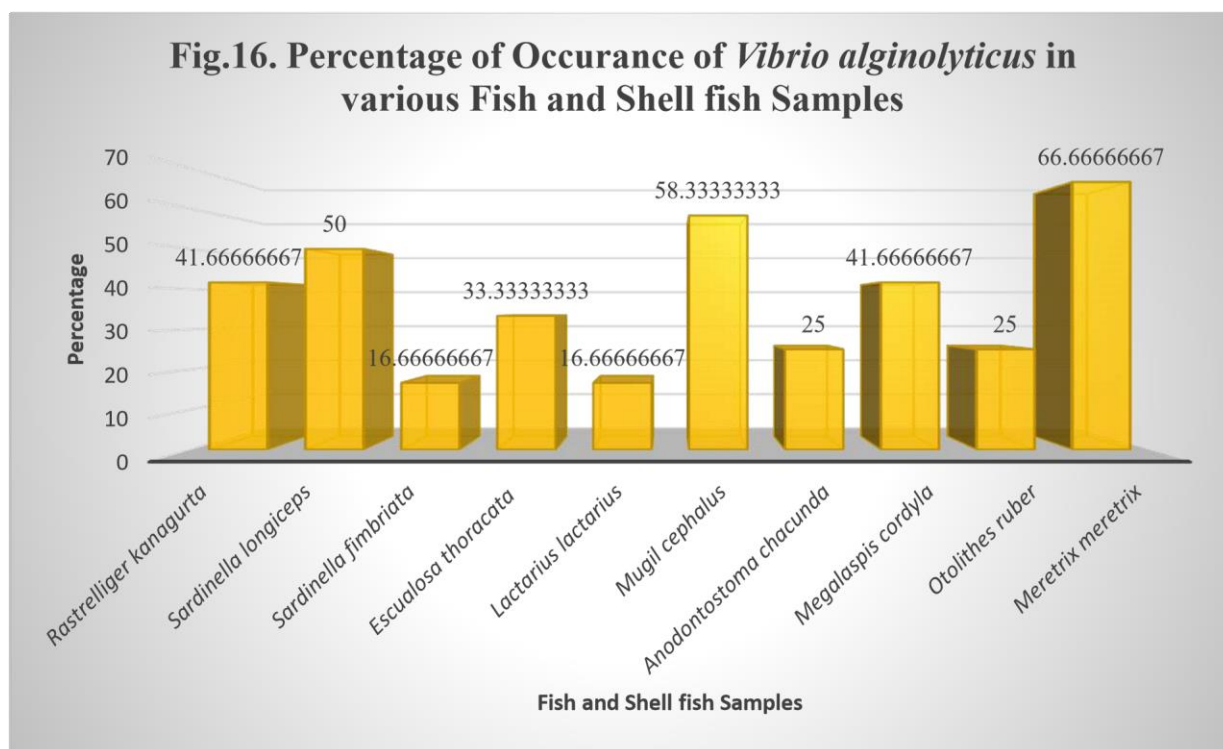


Figure 15 : Image of amplified DNA and Ladder



CONCLUSION

The present study aimed to investigate the prevalence of *Vibrio alginolyticus* in commercially important fish and shellfish. Our findings reveal a significant presence of this pathogen in various seafood species, highlighting potential health risks associated with consuming raw or undercooked seafood. The contamination rates observed in this study varied among different species, with *Meretrix meretrix* exhibiting the highest prevalence. The results suggests that certain species may be more susceptible to *V. alginolyticus* colonization. Moreover, the study underscores the potential for contamination in both pelagic and benthic organisms, emphasizing the need for comprehensive food safety measures. The presence of *V. alginolyticus* in seafood poses a significant public health concern, as this bacterium can cause severe illnesses, including gastroenteritis and septicemia. Therefore, it is imperative to implement stringent food safety practices, such as proper handling, storage, and cooking of seafood, to mitigate the risk of *V. alginolyticus* infections. In conclusion, this study provides valuable insights into the prevalence of *Vibrio alginolyticus* in seafood



and highlights the importance of adopting comprehensive food safety measures to protect public health. Future research should focus on identifying factors that contribute to the variability in contamination rates among different species and developing effective strategies for preventing the spread of this pathogen in the seafood supply chain.

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