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Research Article

Histopathological Alterations in *Meretrix meretrix* Induced by *Vibrio* species

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Abstract: The study was carried out to investigate the histopathological changes in the shellfish samples associated with the *Vibrio* species which are collected from the Kali Estuary and also collected from the Fish market of Karwar. The collected samples were soon processed in lab to avoid the contamination. The *Vibrio* species were isolated and identified from the *Meretrix meretrix* samples using standard microbiological and molecular protocol. The maximum of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* species were isolated and identified in the samples collected from market when compared to wild from the Estuary. The histopathological changes were observed in the gills, mantel and the digestive tissues. Observed alterations included haemocyte infiltration, inflammation, and necrosis within gill lamellae and mantle tissues. This is due to the pathogenic *Vibrio* species.

Keywords: *Meretrix meretrix*, Histopathological changes, *Vibrio parahaemolyticus* and *Vibrio alginolyticus*.

Introduction

Bivalve such as commercially important *Meretrix meretrix*, are integral components of coastal ecosystems, serving as vital links in food webs and are economically important for the coastal community [1]. However, these filter-feeding organisms are constantly exposed to a diverse microbial community, including potentially pathogenic bacteria of the genus *Vibrio*. These species are ubiquitous in marine and estuarine environments, and certain strains are recognized as significant pathogens affecting both aquatic animals and humans [2].

The increase in prevalence of Vibrio infections in shellfish populations has raised concerns about food safety and the health of aquatic ecosystems [3]. Specifically, *Vibrio parahaemolyticus* and *Vibrio alginolyticus* are frequently implicated in shellfish-borne illnesses and are known to cause significant morbidity and mortality in bivalves [4]. These bacteria can induce a range of pathological changes in host tissues, including inflammation, necrosis, and haemocyte infiltration, ultimately compromising the physiological functions of vital organs such as the gills, mantle, and digestive system [5].

Environmental factors, such as temperature, salinity, and nutrient availability, play a crucial role in shaping the distribution and virulence of *Vibrio* species [6]. Anthropogenic activities, including pollution and intensive aquaculture practices, can further exacerbate the risk of *Vibrio* infections in shellfish populations [7]. Therefore, understanding the histopathological alterations induced by *Vibrio* species in *Meretrix meretrix* is crucial for assessing the health status of these valuable resources and for developing effective strategies to mitigate the impact of bacterial infections.

This study aims to investigate the histopathological changes occurring in *Meretrix meretrix* associated with *Vibrio* species, particularly *Vibrio parahaemolyticus* and *Vibrio alginolyticus*, isolated from clams collected from both the wild environment of the Kali Estuary and the commercial setting of the Karwar fish market.

Materials and Methods

1. Sample Collection

Meretrix meretrix samples were collected from two different stations i.e. from Kali estuary and Karwar Fish market.

2. Isolation and Identification of Vibrio Species

The *Meretrix meretrix* tissues, gills, mantle were aseptically collected for the isolation of *Vibrio* spp. The samples were serially diluted in sterile saline solution and then inoculated on TCBS Agar plates and incubated at 37^0C for 24 hrs. The isolated green and yellow colonies on TCBS plates were identified using biochemical and molecular test.

3. Histopathological Analysis

The dissected *Meretrix meretrix* sample of same part were used to study histopathological changes due to pathogenic *Vibrio* species. The tissue samples were fixed in Davidson fixative. The fixed tissues were dehydrated in ethanol gradient and cleared in xylene and embedded in paraffin wax and then sectioned using microtome. Sections were stained with hematoxylin and eosin and fixed with DPX. These slides were observed under microscope [8].

Results

The *Vibrio parahaemolyticus* and *Vibrio alginolyticus* were isolated from the *Meretrix meretrix* from both the stations. The bacterial load in the samples collected from Karwar fish market was observed maximum compared to that of Kali estuary. The isolation and identification of *Vibrio* species from the *Meretrix meretrix* samples revealed a higher prevalence of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* in samples collected from the Karwar Fish market compared to those from the Kali Estuary. Histopathological examination of the *Meretrix meretrix* tissues revealed several alterations, particularly in the gills and mantle, which were associated with the presence of pathogenic *Vibrio* species. The observed changes are haemocyte infiltration, necrosis, spaces between cells. These histopathological alterations, characterized by haemocyte infiltration, inflammation, and necrosis in the gills, mantle, and to a lesser extent, the digestive tissues of *Meretrix meretrix*, are consistent with the pathogenic effects of *Vibrio* species, particularly *Vibrio parahaemolyticus* and *Vibrio alginolyticus*. The more severe alterations observed in clams from the Karwar Fish market correlate with the higher prevalence of these *Vibrio* species in those samples. The study of histopathological changes due to *Vibrio* bacterial effect in the different tissues of *Meretrix meretrix* was studied. The present observations showed the changes in gills and mantle tissue. The necrosis due to *Vibrio* spp. was observed in the gills. It is due to adaptation of bivalves to the filter feeding mechanism, lot of the pathogens attacks gills of the bivalves as they are benthic filter feeding organisms. Necrosis in gills, disorganisation or uneven arrangement of muscle fibres and vacuole formation was observed.

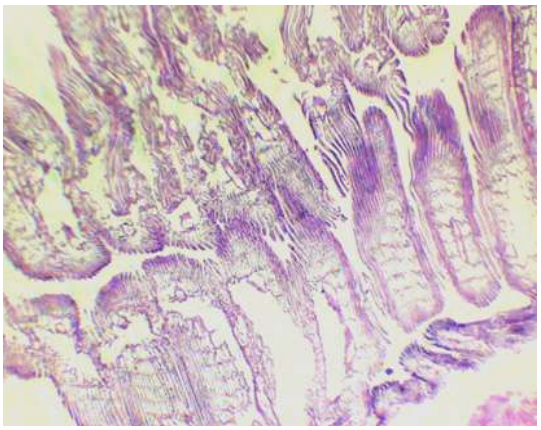

Figure 1: Infected Gill of *M .meretix* (5X; H&E).

Figure 2: Normal Gill of *M.meretrix* (10X; H&E).

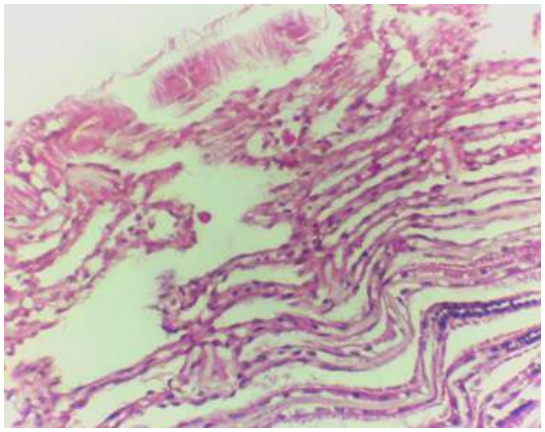

Figure 3: Gills of *M.meretrix* showing Hemorrhage (40X; H&E).

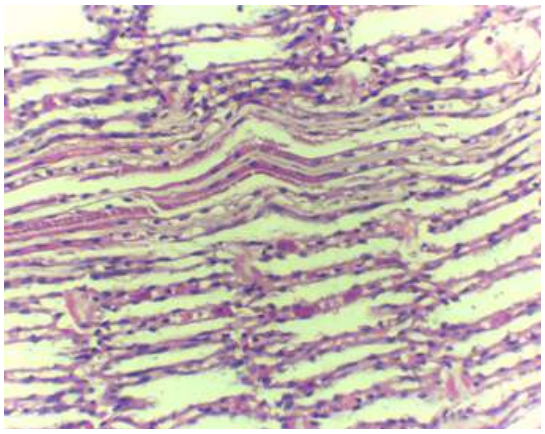

Figure 4: Gill of *M.meretix* (40X; H&E).

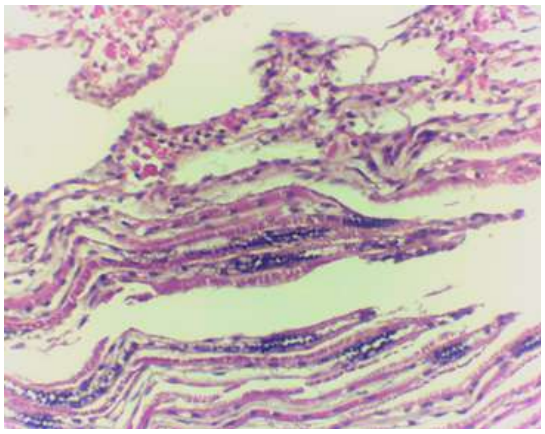

Figure 5: Gill of *M.meretrix* showing Necrosis (40X; H&E).

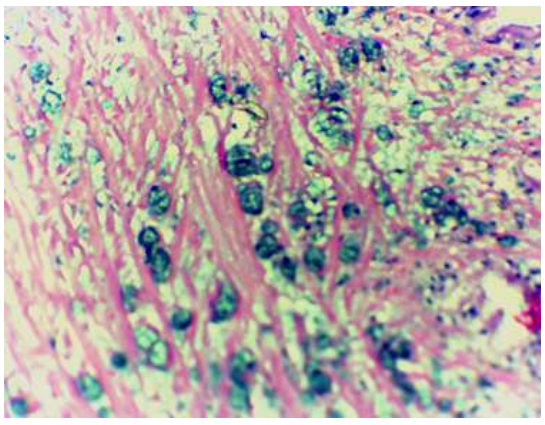

Figure 6: *M.meretrix* tissue showing necrosis (40X; H&E).

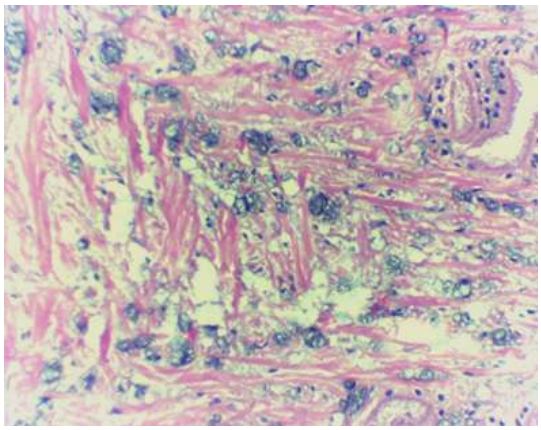

Figure 7: Mantle of *M.meretrix* showing Necrosis (40X; H&E).

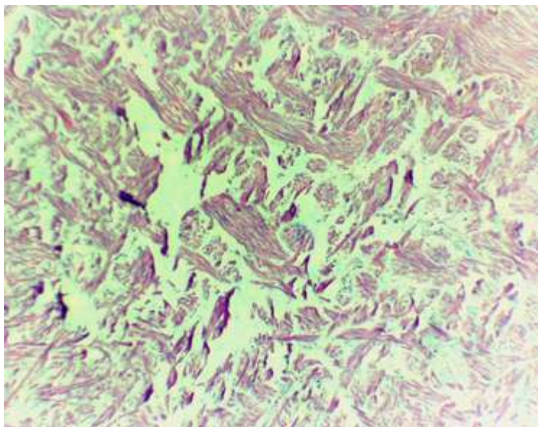

Figure 8: Disintegration of Mantel tissue of *M.meretrix* (10X; H&E).

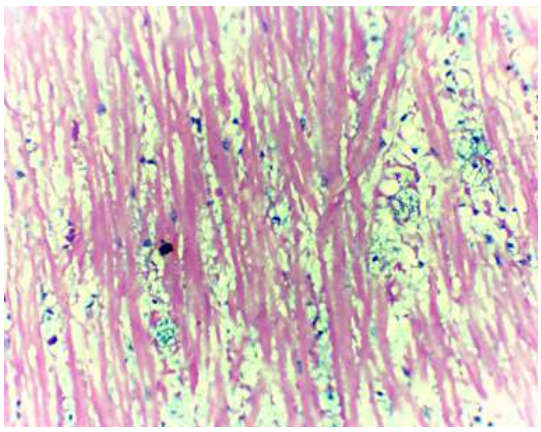

Figure 9: Vacuole in tissues of *M.meretix* (10X; H& E).

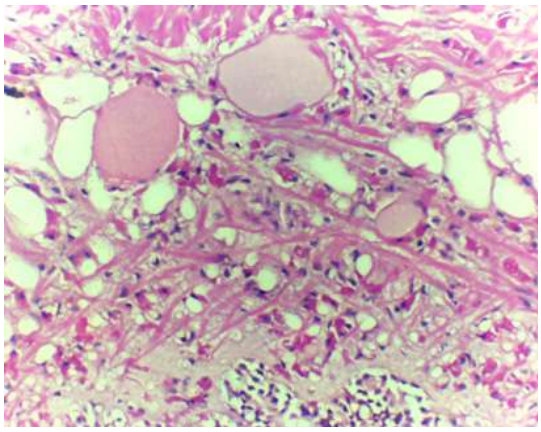

Figure 10: Vacuole in tissues of *M.meretix* (40X; H& E).

Conclusion

The study unequivocally demonstrates a clear association between *Vibrio* species and observed histopathological changes in the shellfish species *Meretrix meretrix*. Specifically, *Vibrio parahaemolyticus* and *Vibrio alginolyticus* were found to be the dominant species, with their prevalence notably higher in samples obtained from the Karwar fish market compared to those from the Kali Estuary, similar observation was observed by Revankar, S. K et.al.,2023. The presence of these pathogenic *Vibrio* species induced significant cellular and tissue damage, characterized by haemocyte infiltration, inflammation, and necrosis in critical organs such as the gills, mantle, and digestive tissues. These findings highlight the potential health risk posed by *Vibrio* contamination in shellfish, particularly those from commercial markets, and underscore the importance of monitoring and managing *Vibrio* species in both wild and commercially sourced bivalves to safeguard both shellfish health and human consumers.

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