"Study of Vibrio spp. and its Pathological changes in the organs of bivalve Meretrix meretrix from Kali and Aghanashini Estuary of Uttara Kannada, Karnataka."

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Abstract

The present study was carried out for the period ten months from September 2021 to June 2022, to isolate and identify the Vibrio spp. and to study the pathological changes in vital organs of bivalve Meretrix meretrix, one of the important edible bivalve of this region. Samples were collected from the two estuaries Kali Estuary (140 80' 58" N and 740 14'26.2" E) and Aghanashini Estuary (14o 52` 25.6``N and 74o 36` 67.5``E) of Uttara Kannada, Karnataka. The present study was carried out to assess the presence of pathogenic Vibrio spp. The bivalve samples were collected in sterile condition and immediately processed for necropsy followed by bacterial isolation and then vital organs were fixed in Davidson fixative to study histopathological changes in vital organs. The Total Vibrio Count was observed in the range of 103 CFU/gm to 104 CFU/gm. The Green and yellow colonies were isolated and purified in TCBS agar. Purified colonies were further identified by using biochemical tests as Vibrio spp. 16S rDNA gene sequence was done for molecular identification and then confirmed as Vibrio parahaemolyticus and Vibrio alginolyticus. During the present study it was found that 50% were V.parahaemolyticus whereas 48% were V.alginolyticus and the remaining 2% constituted other Vibrio spp. Few histopathological changes were also seen in the mantle and gills of the Meretrix meretrix in the current study.

Keywords*: Kali, Aghanashini, Meretrix meretrix, Vibrio parahaemolyticus, Vibrio alginolyticus, Marine Biology.*

Introduction

Bivalve which comes under phylum mollusc and class Bivalvia, are the economically important organisms in the fisheries. Bivalves like clams, mussel and oysters are the filter feeding organisms, they accumulate the large diversity of bacterial flora from the surrounding environment i.e. from water and sediment, which could be infectious to the higher organisms and humans also from the food chain. The bacterial flora include various species belonging to different genera like Vibrio, Photobacterium, Psuedomonas, Moraxella, Aeromonas, Micrococcus, Actinobacter and Bacillus(Cathie S.W. Kueh et al., 1985). Some bacteria may be pathogens in cultured organisms and some may affect in wild organisms. The most bacterial disease in

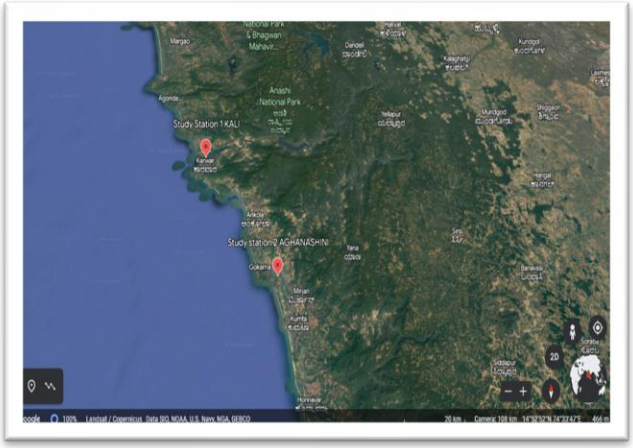
bivalve are caused by gram negative bacteria i.e. Vibrio spp, Psuedomonas and Aeromonas spp. These bacterial species cause necrosis in bivalve. Vibrio spp can produce exotoxin, ciliostatic factors which causes deciliation, loss of velar epithelium and abnormal swimming behaviour (E Valério et al., 2010). Vibrio parahaemolyticus and Vibrio alginolyticus are the pathogens for the shellfishes. Usually these species are mainly found in estuarine and marine environment, in free swimming and its motility is confined with a single polar flagellum, which can inert and intimate in the surfaces of zooplanktons, fish, shellfishes and mainly in bivalves which are suspended in the sediments (Gode-Potratz et al., 2011 and V.letchumanan et al.,2014). These causes the gastrointestinal disorders mainly transmitted due to the consumption of raw or uncooked food (Newton et al., 2013), ear, wound infections and septicaemia which are life threatening to the patients with illness (L zang et al.,2013). Shell fishes are considered as poor mans rich protein food of majority of inhabitants of coastal area. Bivalves are usually consumed by local public and even it is transported to different places. In the present study an attempt is made to isolate and identify the pathogenic Vibrio spp from one of the important edible bivalve Meretrix meretrix from two estuaries of Uttara Kannada district Karnataka.

Materials and Methods

Study Area

Two estuaries of Uttara Kannada district Karnataka i.e. Kali Estuary and Aghanshini Estuary selected for present work. Kali Estuary (14080’58”N & 74014’26.2”E) located in Karwar taluka. Kali river arises from the place called Diggi Joida Taluka. It flows to south west into Kadra Reservoir, and is joined by the Thana Halla just below the dam at Kadra. From Kadra the river flows west through marshland to join the Arabian sea in Karwar.

Figure.1: Map showing Study Stations



The Aghanashini or Tadri River (14052’25.6”N& 74036’67.5”E) originates in the Sirsi taluk of Uttara Kannada district in the central Western Ghats of Karnataka State. The river meets the tides of the Arabian Sea and forms a large estuarine expanse in the coastal taluk of Kumta. The estuary has its outlet into the sea in between the villages of Aghanashini in the South and Tadri in the North.

Sample Collection & Analysis

Bivalves (Meretrix meretrix) were collected monthly from both the estuaries i.e. Kali and Aghanashini estuary for ten months (September 2021 to June 2022). M. meretrix samples were collected during low tide in sterile polythene bags, and were immediately transported to lab in aseptic condition for further analysis.

Figure.2: Meretrix meretrix

Sample Preparation

The bivalve tissue samples were aseptically dissected to cut open the shell and the tissue sample is aseptically taken out in sterile mortar. The sample was then homogenized in sterile PBS solution; it is taken as 1:10 dilution. After thoroughly mixing the homogenate by vortex for 2 mins, 1 ml of homogenate was transferred to 9ml of dilution to get 1:100 dilutions. Similarly required dilutions are prepared. 0.5 ml of each required dilution were spread plated on sterilized TCBS Agar plates in duplicate. The inoculated plates were incubated in 370C for 24 hrs. The colonies falling in between the range of 30-300 were selected for calculating total vibrio count.

The obtained green and yellow colonies on TCBS agar plates are then purified on TSA plates and the biochemical test were carried out (Noguerola and Blanch 2008). DNA of Vibrio were isolated for molecular identification. 16S rDNA gene was amplified using 27F and 1492R primers. The 16S rDNA gene sequence was used to carry out BLAST with the database of NCBI genbank database.

Histopathology

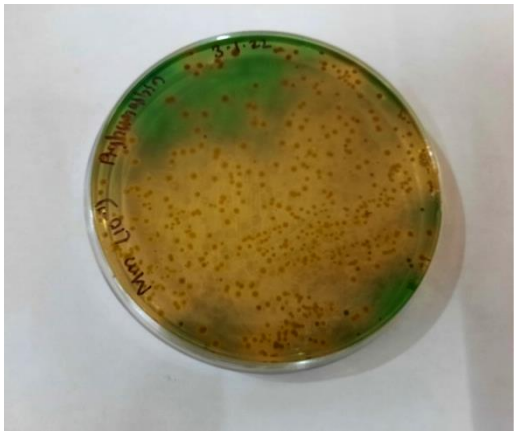
Tissue samples of Meretrix meretrix were fixed in Davidson's fixative (B Shawl et al., 1957) for 48 hrs and then preserved in ethanol 70%. Before sectioning the tissues were dehydrated in ethanol series, embedded in wax, preparation of blocks for sectioning, sectioning and staining with haematoxylin and eosin stain. The slides were observed under microscope to study the pathological changes.

Results and Discussion

The Total Vibrio Count in M.meretrix of Kali and Aghanashini estuary was observed in the range of 103 to 104. The Vibrio load greater than 105 can serious damage and infection (FSANZ 2018). The highest count of Vibrio spp. was found in M.meretrix of Aghanashini i.e 9.6X104 and lowest was 6.0X103 whereas the highest count in Kali estuary was 2.8X104 and lowest 7.3X103 CFU/gm. The similar kind of observation was observed by Haragi, Shivakumar et al., 2016 in Paphia malabarica of Kali estuary.

The prevalence of the Vibrio parahaemolyticus and Vibrio alginolyticus in M.meretrix of both the estuaries was calculated. Total 120 samples from both the estuaries were collected, 60 samples from each estuary. 36 samples of Kali estuary was positive for V.parahaemolyticus (60%) and 21 samples were positive for V.alginolyticus (35%); whereas in Aghanashini estuary 23 samples were positive for V.parahaemolyticus (39%) and 36 samples were positive for V.alginolyticus (60%). Out of 120 samples 50% were positive for V.parahaemolyticus whereas 48% were positive for V.alginolyticus and the remaining 2% constituted other Vibrio spp.

Figure 3 and 4: Isolated Vibrio spp.



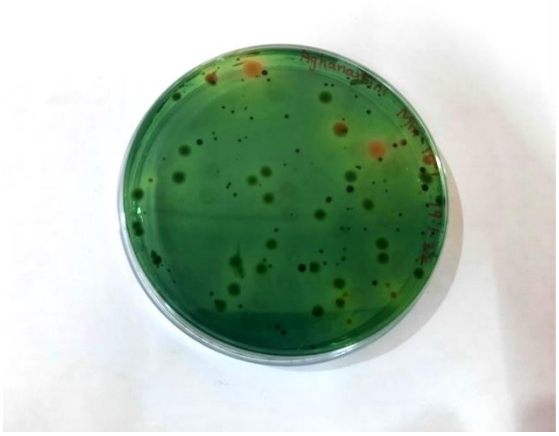
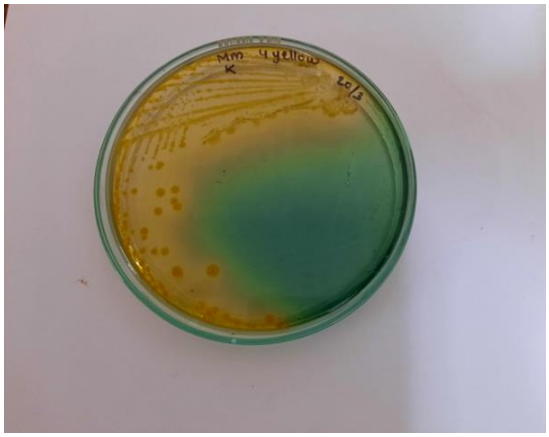


Figure.5: Pure colonies of V.parahaemolyticus

Figure.6: Pure colonies of V.alginolyticus



The 16S rDNA sequence which was BLAST with the data base of NCBI gene data base, the result for these samples showed similarities (99.55%) with V.parahaemolyticus and (99.40%) V.alginolyticus based on nucleotide homology and phylogenetic analysis.

Figure 7: Amplification of gene of Vibrio parahaemolyticus from bivalve

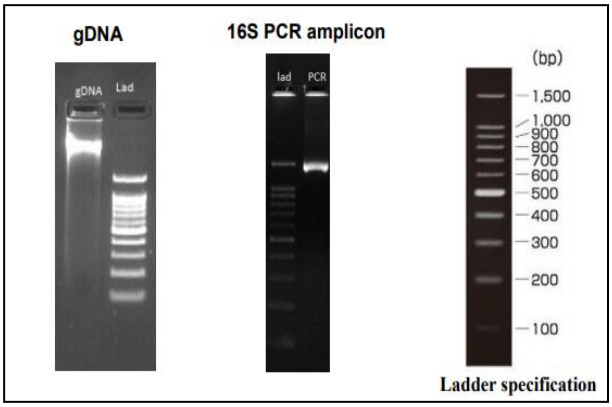


Figure 8: Amplification of gene of Vibrio alginolyticus from bivalve

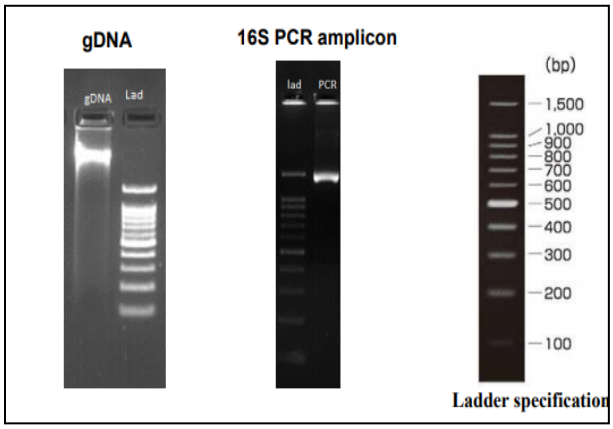


Figure 9: Distance tree based on fast minimum evolution using BLAST showing Vibrio parahaemolyticus

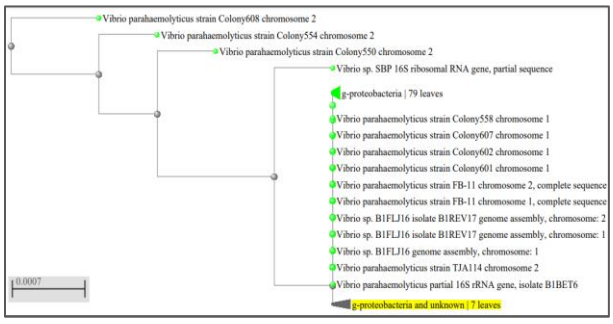
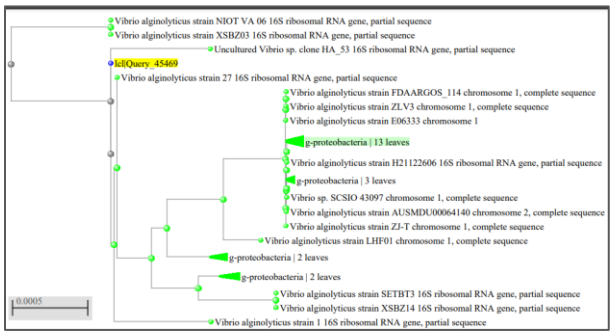


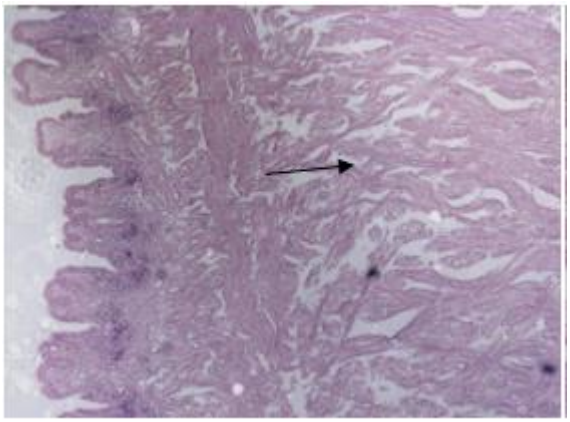
Figure 10: Distance tree based on fast minimum evolution using BLAST showing Vibrio alginolyticus



Shell fishes and bivalves are more prone to tissue contamination due to the microorganisms from the marine environment, as these bivalves are the filter feeders, they filter large volume of water. During this process, these bivalves accumulate the pathogenic bacteria like Vibrio spp. which is present in water and sediment. Thus, it can concentrate in tissues of these bivalves and causes ill effects (M.M Mousa et al.,2019).

Few histopathological changes were observed in gills and mantle tissue of M.meretrix due to V.parahaemolyticus and V.alginolyticus. Histological examination revealed that the tissue necrosis, disorganisation of mantle cells and folds in mantle tissue was observed. Similar kind of observation was noticed by J Gomez-Leon et al., 2005, authors observed the bacillary bacterial infection with necrosis in clam tissue, pale colour digestive tract infection and bacillary bacteria were found along the mantle fold. Present findings were corroborated with the results of C.Zannella et al.,2017, who has reported Vibrio spp. can cause tissue necrosis due to the production of exotoxin by bacteria.

Figure 11 and 12: Black arrow shows disorganization of mantle tissues in M.meretrix (H&E;50x)



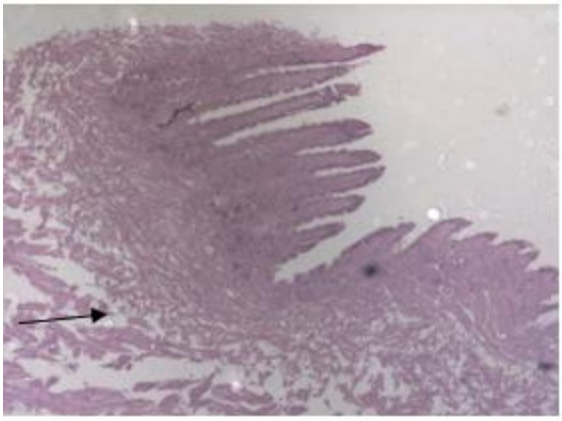
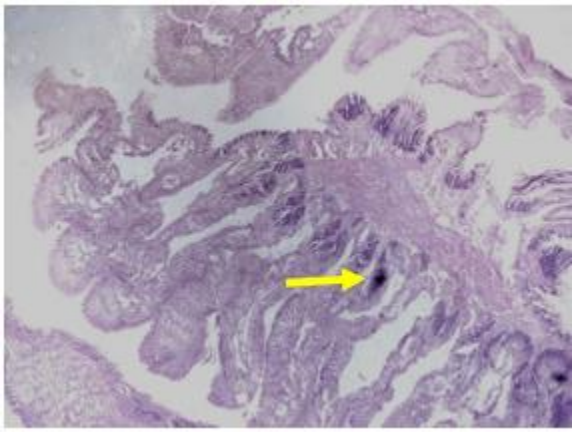


Figure 13 and 14: Yellow arrow shows necrosis of the of gill tissues of M.meretrix (H&E;50x)



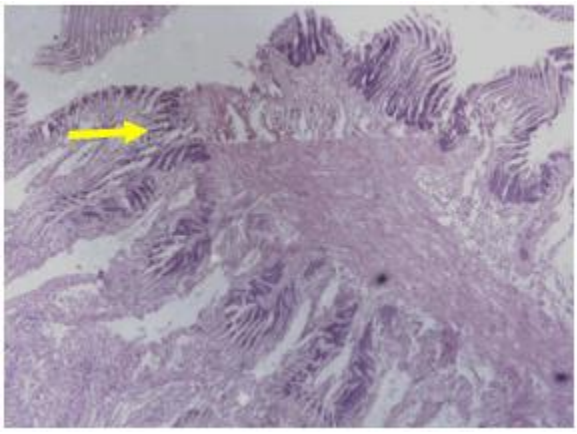
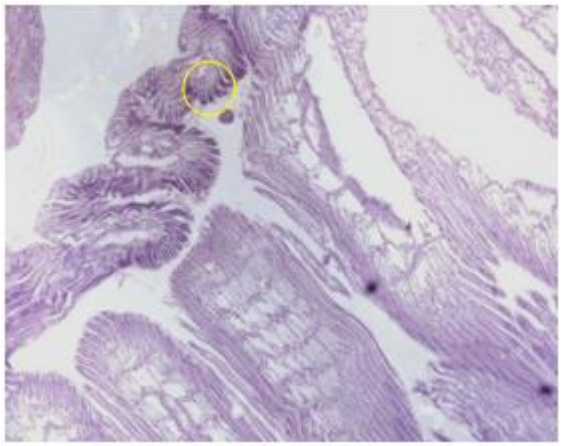
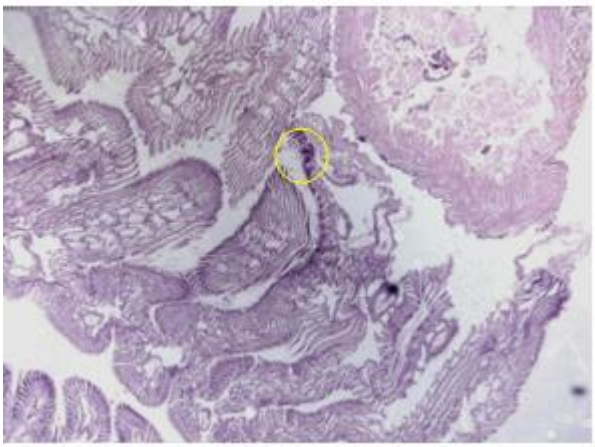


Figure 15 and 16: Pathological changes yellow circle shows necrosis of the of gill tissues of M.meretrix (H&E;50x)





Conclusion:

The present study was carried out to identify and isolate the pathogenic Vibrio parahaemolytics and Vibrio alginolyticus from one the commercially important edible bivalve Meretrix meretrix from two estuaries Kali and Aghanashini of Uttara Kannada Karnataka, and also to study the pathogenic changes in the tissues of the bivalve. The pathological changes like tissue necrosis due to Vibrio spp can cause mortality in bivalves. If pathogenic Vibrio spp. load is greater than 105 pathogenic cells (FSANZ 2018), can cause serious infection, which include bloody diarrhoea, abdominal pain, nausea and vomiting. The

best way to control the pathogenic bacterial load is to store the shell fishes or bivalves in 50C immediately after harvest. When the Consumption of raw or partially cooked foods, contaminated with these pathogenic bacteria can cause illness to the consumers, in order to avoid this effects it is advised that the food should be well cooked before consumption.

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