Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 7 Issue 3 March 2024

Research Article

Screening of Pathogenic Bacteria from the Commercially Important Edible Bivalve *Meretrix meretrix*

Sujal K Revankar* and J L Rathod

Department of Studies in Marine Biology, Karnatak University Post Graduate Centre Karwar, Karnataka, India

***Corresponding Author:** Sujal K Revankar, Department of Studies in Marine Biology, Karnatak University Post Graduate Centre Karwar, Karnataka, India.

DOI: 10.31080/ASMI.2024.07.1357

Received: February 01, 2024

Published: February 20, 2024

Abstract

Bacteria widely occur in aquatic environment in marine, estuarine and they are the pathogens to the aquatic organisms. Pathogenic bacteria mainly include *Vibrio* Species, Pseudomonas, Aeromonas, *Staphylococcus* etc. Vibrio species are the casual agents of zoonosis, which causes gastrointestinal disorders and septicaemia. The study was carried out for the screening of pathogenic Vibrio species in the edible bivalve *Meretrix meretrix* and also from its habitat. *M.meretrix* is the benthic, filter feeding organism by which it will accumulate large groups of bacterial flora from the surrounding environment. The *M. meretrix* and sediment samples from Kali estuary was screened for the pathogenic Vibrio load on the TCBS Agar medium. The average pathogenic bacterial load was 4.1 Log CFU/g in sediment whereas in *M.meretrix* it was 4.2 Log CFU/g. The total of 86 *Vibrio* isolates comprising of Vibrio parahaemolyticus (n = 44), Vibrio alginolyticus (n = 30) and Vibrio navarrensis (n = 12) were found. These *Vibrio* species were tested for the antibiotic resistance, showed 100% resistant to ampicillin and ceftotaxime, 95% were resistant to ceftazidime and 75% were resistant to cefepime where as 90% were sensitive to chloramphenicol followed by 80% towards tetracycline and 72.5% towards gentamicin. The present findings showed that presence of *Vibrio* species in estuarine sediments, have affected the *M.meretrix* of the habitat. The presence of antibiotic resistant *Vibrio* species causes ill effects in the organisms causing histopathological changes in affected areas and also humans after the consumption.

Keywords: Kali Estuary; Pathogens; *Vibrio*; Antibiotics; *Meretrix meretrix*

Introduction

Estuaries are incredibly active and productive regions where the river and marine habitats meet. Consequently, a significant amount of the water flowing through a catchment is received by and processed in estuarine and coastal areas. Importantly, estuaries are sites of important aquatic resources that offer food and habitat for fish and shellfish as well as food for humans, as well as locations for tourism and recreation and a variety of other ecosystem services. Urban and agricultural runoff, sewage, toxins, and other direct and diffuse inputs of pollutants to the estuary and coastal zone all have a substantial impact on these ecologically and economically critical places [1-3]. As *Vibrio* bacteria is commonly found in aquatic environment, most bacteria have been observed in fin fishes and shell fishes, most abundantly in shellfishes such as oysters, clams, etc. These bacteria are thus considered to be the natural microflora of the surrounding. Thus, the pathogenic strains arise due the environmental concerns such as increase in the temperature or pollution in the surrounding due to human intervention. It has been reported in studies that during extreme weather conditions, summer, *Vibrio* have been even found in the sediment surrounding the organism as it is contains significant amount of organic matter. *Vibrio* infections are usually caused due to improper eating practice of sea as most are foodborne pathogens. It is also been observed in reports that these pathogenic strains of *Vibrio* are able to grow at an exponential rate due to the presence of Sodium Chloride (NaCl), so its notable presence in the brackish or estuarine water has been seen. They are able to withstand high pH and salinity. Most *Vibrio* spp. Are able to oxidize and ferment glucose without production of gas [4].

Pathogenic *Vibrio* infections are usually related to undercooked or uncooked seafood, thus causing diarrhea with different gastric

related symptoms. Some *Vibrio* species are found in fresh water ecosystem, which show infections in the nearby areas with human habitation, for example, *Vibrio* cholerae. Infections caused by *Vibrio* bacterium is more prevalent in the rural coastal areas where poor hygiene is one of the major factors responsible. Along with causing infections in human it is also been reported that *Vibrio* infections in other marine organisms [5-8].

Important species of *Vibrio* that are pathogenic to humans are *V. cholerae, V. parahaemolyticus, V. vulnificus*. These are potential to cause distress in humans and contaminants in seafood and associated with the consumption of raw or uncooked fish or shellfish. Out of these *Vibrio* species, *Vibrio* parahaemolyticus is the most common source of seafood borne pathogenic infections in humans as well as some marine mammals but *Vibrio* vulnificus has been found to be most lethal [9]. *Vibrio* outbreaks in a particular area (epidemic) is due to the *Vibrio* cholerae, when the animal or human consume contaminated food or water. Most of the ingested bacteria are destroyed by the gastric juices. The bacteria that survive the juices enter in the intestine and form colonies there. These colonies in the intestine cause infection in the organism causing stomach ache and watery diarrhea. According to various reports and studies, it has been estimated there are around 2 to 4 million cholera infections annually causing 100,000 to 300,000 deaths [12]. Other species that have been isolated from the people suffering with gastroenteritis were *V. alginolyticus, V. fluvialis, V. furnissii, V. hollisae, V. metschnikovii and V. mimicus*. These *Vibrio* species occasionally are referred to as pathogenic humans [9].

Diseases caused to human by pathogenic bacteria of *Vibrio* genus are divided into two group: Cholera and Non- Cholera infections. *V. cholerae* is the aetiological agent of Cholera. In cholera infection, a severe watery diarrhoea is caused by ingestion of contaminated food or water. There is also a possibility of person-to-person transmission. *V. cholerae* can also be found in freshwaters unlike other *Vibrio* species. *V. parahaemolyticus and V. vulnificus*, belong to the non-cholera group of pathogens that cause *Vibrio*sis. Ingestion of Non-cholera bacteria can cause mild infections and symptoms of Gastroenteritis or Primary Septicaemia (caused due to ingestion of raw or undercooked contaminated food). Exposure of skin wounds to contaminated water can also cause wound infection that can result in Secondary Septicaemia. Non-cholera bacteria are found in salinity range from high to moderate and can be found in sea food and sea habitats. These bacteria are most important pathogens present in the environment that originate from aquatic and marine habitat. The present study was carried out to screen the pathogenic bacterial *Vibrio* species in the *Meretrix meretrix* and their habitat i.e. Sediment of Kali Estuary.

Material and Methods

Sample Collection: The commercially important edible bivalve sample (*Meretrix meretrix*) and Sediment samples were collected from Kali Estuary ($14^0$50'20" N and $74^0$08'23" E), Uttara Kannada District Karnataka, during low tide. The samples were collected aseptically in sterile polythene bags and then processed in lab.

Figure 1: Collection of Bivalves and Sediment during Low Tide.

Sample Preparation and Analysis: Sediment samples were suspended in distilled water to obtain 1:10 (w/v) dilution, vortexed to mix well; the supernatant fluid was used for analysis. 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 37^0C for 24 hrs. Counted the colonies for calculating total *Vibrio* count.

Identification of Bacterial Samples: The isolated bacteria were studied for the morphological, physiological and biochemical char-

acteristics. For the identification of bacteria different types of tests were carried out like Gram's staining, motility, catalase, oxidase, MR VP, Indole production, citrate, carbohydrate fermentation test for glucose, sucrose, lactose, manitol, maltose, arabinose etc, salt tolerance test (0%, 3%, 6%, 8% and 10%) and urease test. These tests were followed from the scheme for identification of *Vibrios* to species level given by Noguerola and Blanch (2008) [10]. 16S rRNA sequencing of *Vibrio* species was amplified using 27F and 1492R primers. Then the sequence was blasted using NCBI.

Antibiotic Sensitivity Test: The *Vibrio spp.* were tested for antibiotic sensitivity against different antibiotics. The previously identified *Vibrio parahaemolyticus, Vibrio alginolyticus* and *Vibrio navarrensis* were tested on Muller Hilton Agar using eight different antibiotics (ampicillin, cefepime, cefotaxime, ceftazidime, gentamicin, tetracycline, ciprofloxacin and chloramphenicol) by Disc Diffusion method. The test plates were incubated at 37^0C for 24 hrs and the plates were observed for the zone of inhibition surrounding the antibiotics. The diameter of the zone of inhibition was measured using ruler, by placing the petriplate over the dark background. The diameters of zone of inhibition was noted for each antibiotics. The results were interpreted with the guidelines of the Clinical and Laboratory Standards Institute M45-A2 [11]. Based on the observed results bacteria was classified into resistant, sensitive and intermediate to antibiotics.

Results

The screening of pathogenic *Vibrio* from *Meretrix meretrix* and Sediments of Kali Estuary was carried out from Dec 2021 to May 2022 for the period of six months. Total Pathogenic *Vibrio* Load was observed in the range of 3.86 to 4.62 Log CFU/G in *M. meretrix* and 3.86 to 4.57 Log CFU/G in Sediment. The highest *Vibrio* load was Observed in Jan 2022 in *M. meretrix* 4.62 Log CFU/G whereas in Sediment it was 4.57 Log CFU/G in March 2022. The lowest load was 3.86 Log CFU/G in January 2022 in Sediment whereas in May 2022 in *M. meretrix.* Similar kind of observation was made in *Meretrix meretrix* of Kali and Aghanashini estuary by [14].

Total 86 number of *Vibrio* isolated were isolated and from the sediments and *M. meretrix* in TCBS Agar plates with different morphological characters. These isolates were purified on TSA agar plates containing 3% NaCl because *Vibrio*s can grow in high saline (2-10%NaCl). The isolates were biochemically tested with different tests (Catalase test, Indole test, Citrate test, Urease test, Salt tolerance test, Carbohydrate Fermentation test, Temperature test) and were confirmed to be *Vibrio parahaemolyticus, Vibrio alginolyticus* and *Vibrio navarrensis.* The 16S rRNA gene sequencing identified 86 *Vibrio* species of which 3 species were obtained. 51.14% were *Vibrio parahaemolyticus,* 34.88% *Vibrio alginolyticus* and 13.98% *Vibrio navarrensis.* The dominant species was *Vibrio parahaemolyticus* in both sediment and *M. meretrix* whereas, *Vibrio navarrensis* was only observed in the sediments.

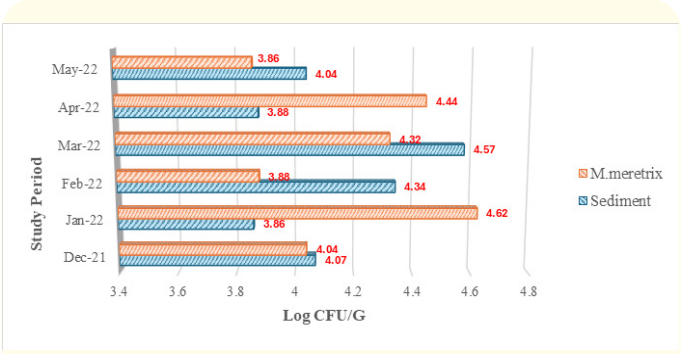


Figure 2: Pathogenic Bacterial Load in *Meretrix meretrix* and Sediment.

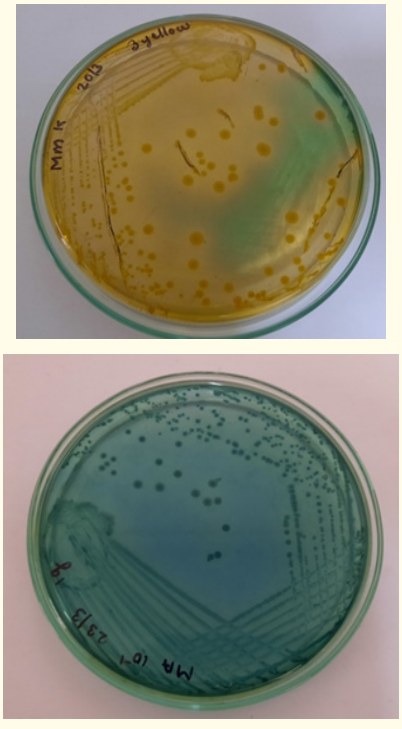


Figure 3: Purified *Vibrio* isolates on TCBS Plates.

The Antibiotic Sensitivity Test were carried out for (n = 44) *Vibrio parahaemolyticus*, (n = 30) *Vibrio alginolyticus* and (n = 12) *Vibrio navarrensis* isolates against eight different antibiotics. All

the *Vibrio* species were resistant to Ampicillin and sensitive toward Chloramphenicol and Tetracycline. *Vibrio parahaemolyticus* showed highest resistance to Ampicillin, Ceftazidime, Cefotaxime and Cefepime antibiotics, where as it was sensitive to Chloramphenicol, Tetracycline, Gentamicin and Ciprofloxacin. *Vibrio alginolyticus* showed highest resistance to ampicillin and cefotaxime, followed by ceftazidime and cefepime. Whereas, it was sensitive against tetracycline, chloramphenicol and gentamicin. *Vibrio navarrensis* was resistant and intermediate resistant to ampicillin, ceftazidime, cefotaxime and cefepime antibiotics. Whereas it was sensitive against chloramphenicol, gentamicin and tetracycline. Similar kind of observation was done by [15] *Vibrio* species isolated from the sediments of Kali and Aghanashini estuary. The pathogens have been become resistant toward the antibiotics due to the excess use of antibiotics in different mariculture activities and even due to anthropogenic activity these antibiotics accumulated in the estuarine environment, the microbes get resistance toward these from the external environment.

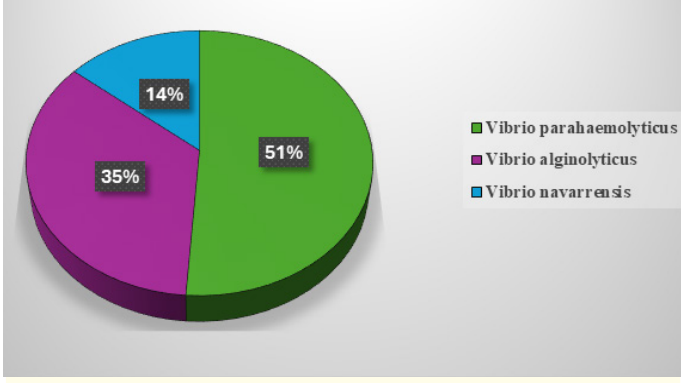
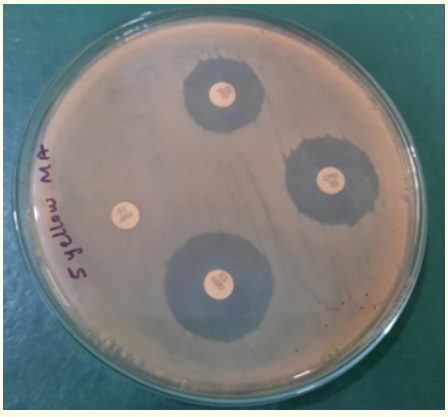


Figure 3: Prevalence of Vibrio species in Kali Estuary.



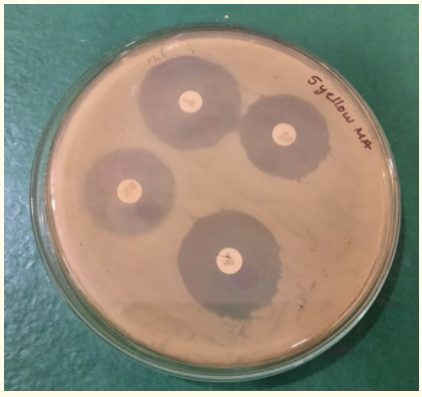


Figure 5: Image showing Zone of Inhibition.

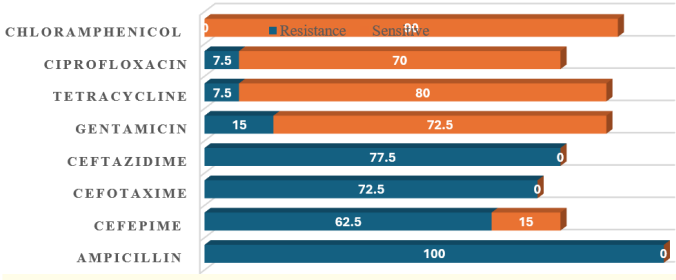


Figure 6: Percentage of *Vibrio* parahaemolyticus showing tolerance to antibiotics

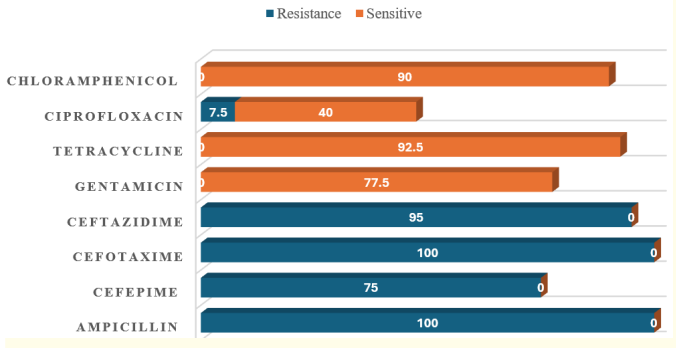


Figure 7: Percentage of Vibrio navarrensis showing tolerance to antibiotics.

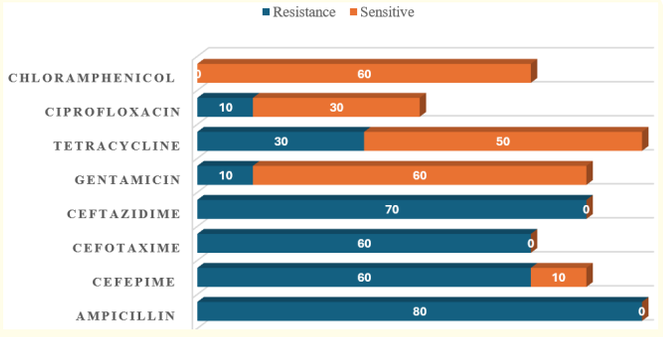


Figure 8: Percentage of *Vibrio* navarrensis showing tolerance to antibiotics

Conclusion

The contamination of pathogenic bacteria causes illness in infected organisms including humans. *Vibrio* species in estuarine environment are common due to the anthropogenic source. The pathogenic load greater than 10^6 can cause serious infection which include bloody diarrhoea, abdominal pain, nausea and vomiting [13]. In the present study *Vibrio parahaemolyticus, Vibrio alginolyticus* and *Vibrio navarrensis* were identified from Sediment and *Meretrix meretrix* of Kali Estuary Karnataka. *Vibrio parahaemolyticus* and *Vibrio alginolyticus* were observed in both sediment and *M. meretrix* whereas *Vibrio navarrensis* was only isolated from the sediments. The *Vibrio species* were resistant to Ampicillin, Ceftazidime, Cefotaxime and Cefepime antibiotic where as they were sensitive to Gentamicin, Tetracycline, Chloramphenicol and Ciprofloxin. The pathogenic bacterial contamination was higher in *M. meretrix* compared to that of sediments. The bivalves have the capability to bioaccumulate pathogens from the external environment as they are the filter feeders. The best way to control the bacterial load is to store the shell fishes in 5^0C immediately after harvest. The consumption of raw or partially cooked food, contaminated with pathogenic bacteria can cause illness to consumers, the best way to avoid is to cook food properly.

Bibliography

1. O'Higgins Timothy G., *et al*. "Habitat scale mapping of fisheries ecosystem service values in estuaries". *Ecology and Society* 15.4 (2010).

2. Savage Candida., *et al*. "Ecosystem services transcend boundaries: estuaries provide resource subsidies and influence functional diversity in coastal benthic communities". (2012): e42708.

3. Costanza Robert, *et al*. "The value of the world's ecosystem services and natural capital". *Nature* 387.6630 (1997): 253-260.

4. Baumann Paul. "Family II. Vibrionaceae". *Bergey's Manual of Systematic Bacteriology* 1 (1984): 516-550.

5. West PA. "The human pathogenic vibrios–A public health update with environmental perspectives". *Epidemiology and Infection* 103.1 (1989): 1-34.

6. Janda J Micheal., *et al*. "Current perspectives on the epidemiology and pathogenesis of clinically significant Vibrio spp". *Clinical Microbiology Reviews* 1.3 (1988): 245-267.

7. Horseman Michael A and Salim Surani. "A comprehensive review of Vibrio vulnificus: an important cause of severe sepsis and skin and soft-tissue infection". *International Journal of Infectious Diseases* 15.3 (2011): e157-e166.

8. Bell Andrew and Michael Bott. "Vibriosis:: what you and your patients need to know". *Delaware Journal of Public Health* 7.1 (2021): 14.

9. Charles Richelle C., *et al*. "Humans surviving cholera develop antibodies against Vibrio cholerae O-specific polysaccharide that inhibit pathogen motility". *MBio* 11.6 (2020): 10-1128.

10. Noguerola I and A R Blanch. "Identification of Vibrio spp. with a set of dichotomous keys". *Journal of Applied Microbiology* 105.1 (2008): 175-185.

11. Jorgensen, James H. "Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guideline". *(No Title)* (2010).

12. Mead, Paul S., *et al*. "Food-related illness and death in the United States". *Emerging Infectious Diseases* 5.5 (1999): 607.

13. FSANZ. Compendium of Microbiological Criteria for Foods (2018).

14. Revankar Sujal K and J L Rathod. "Study of Vibrio spp. and its Pathological changes in the organs of bivalve Meretrix meretrix from Kali and Aghanashini Estuary of Uttara Kannada, Karnataka". *Journal of Survey in Fisheries Sciences* 10.3S (2023): 5723-5730.

15. Revankar S K and J L Rathod. "Antibiotic Resistance in the Vibrio Species Isolated from the Estuarine Sediments of Uttara Kannada Karnataka". *International Journal of Membrane Science and Technology* 10.2 (2023): 4274-4279.