



ISOLATION OF SPECIFIC PATHOGENIC BACTERIA *VIBRIO PARAHAEMOLYTICUS* FROM SHELLFISHES OF KALI ESTUARY, KARWAR, KARNATAKA

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ABSTRACT

Vibrio parahaemolyticus (*V.p.*) occur in marine environments such as inshore, estuarine and backwaters along the coast and causes gastrointestinal illness in humans. Shellfishes particularly filter feeders are able to acquire *V. parahaemolyticus* from its surrounding habitat. The present study was conducted to trace *V. parahaemolyticus* in the commercial shellfishes along the Kali estuary, Karwar. Commercially important and edible shellfishes like, *Crassostrea madrasensis*, *Paphia malabarica* and *Fenneropenaeus (penaeus)* species were selected for this study. The presence of *V. parahaemolyticus* traced in all these shellfishes, of which *C. madrasensis* showed its presence throughout the study period. In *P. malabarica* presence of *V. parahaemolyticus* observed in two seasons, winter and summer but the dominating numbers were in summer. Similarly in *F. indicus*, presence of *V. parahaemolyticus* traced only during the summer season indicating strong association with the temperature. *V. parahaemolyticus* has been considered as major cause of food borne illness and its presence in the shellfish's of Kali estuary indicating the precautions to be taken while consuming raw or uncooked sea foods.

KEY WORDS: Shrimp; Bivalve; Oyster; Marine; Seafood.

INTRODUCTION

Bacterium are minute living microorganisms measuring micrometers in length, found in all kind of environment, some are aerobic whereas other anaerobic. Mainly bacterium are free-living but some are symbionts, exist together in millions (Robert, 2009). Bacterium found in three types spherical, rod and spiral shaped. *Vibrio parahaemolyticus* of family *Vibrionaceae* is a gram negative bacteria which is halophilic, non-spore forming, curved rod shaped bacteria. Its width is 0.5-0.8 μ meter and length about 1.4-2.4 μ meter as described by Fujino (1953). This bacterium is a facultative anaerobe that can ferment glucose without gas production. Having flagella, helps in migration in semisolid surface by "swarming" high motility in liquid media (Dalmin *et al.*, 2002). Presence of this bacterium shows green-blue colonies in Thiosulfate Citrate Bilesaltes-Sucrose agar media (TCBS). *V.p.*, is widespread all along the coastal marine waters and cosmopolitan in distribution. The organism considered common inhabitant of estuaries being found all through the estuarine environment and food chain like, Water, sediment, plankton, fish & shellfish and prevalent during warm season (Joseph *et al.*, 1982). Primary transmission is through ingestion of raw undercooked food or contaminated shellfishes such as shrimps, oysters and bivalve (Annie and Marta 2012). Extremely sensitive to heat or no longer detected at 48°C -50°C after 5 minutes, therefore proper cooking is must for the consumption. By cold storage at -18°C or - 24°C for 15-18 weeks cells can

completely inactive (Su & Liu 2007). Salinity plays crucial for its growth and multiplication. Shrimps infected with large scale of *V. parahaemolyticus* shows eye opacity & protrusion, liquid or blood protrusion and weakness with weak movements, pale gill and anorexia are reported (FAO 2013). This pathogen is a widespread source of food borne illnesses in several Asian countries, including China, Japan and Taiwan, and is recognized as the leading cause of human gastroenteritis associated with seafood consumption in the United States (Daniels *et al.*, 2000). There are abundant data available on *V. p.*, food poisoning in humans through the contaminated sea foods from the eastern Asian country, but very few literatures available in Indian coastal waters. Domestic fish market survey in southwest coast of India for microbial quality has revealed that 81% of the sea foods samples were exceed with *V. parahaemolyticus* (Rekha *et al.*, 2008). Lack of such studies from our region prompted us to uptake the present study and it is first of its kind conducted along the Kali estuary, Karwar, West coast of India with the objective of isolation, identification and to observe seasonal presence of specific pathogenic bacteria *Vibrio parahaemolyticus* from commercially important shell fishes.

MATERIALS & METHODOLOGY

Kali estuary, Karwar, West coast of Karnataka (14°52'N latitude and 74°04'E longitude), is highly productive biotope forms wetland and mangrove ecosystems along the bank of

estuary (Fig. 1). Besides it provides adequate area for intensive aquaculture especially shrimps, moreover supports

the traditional fishing activity as revenue sources for the stakeholders.

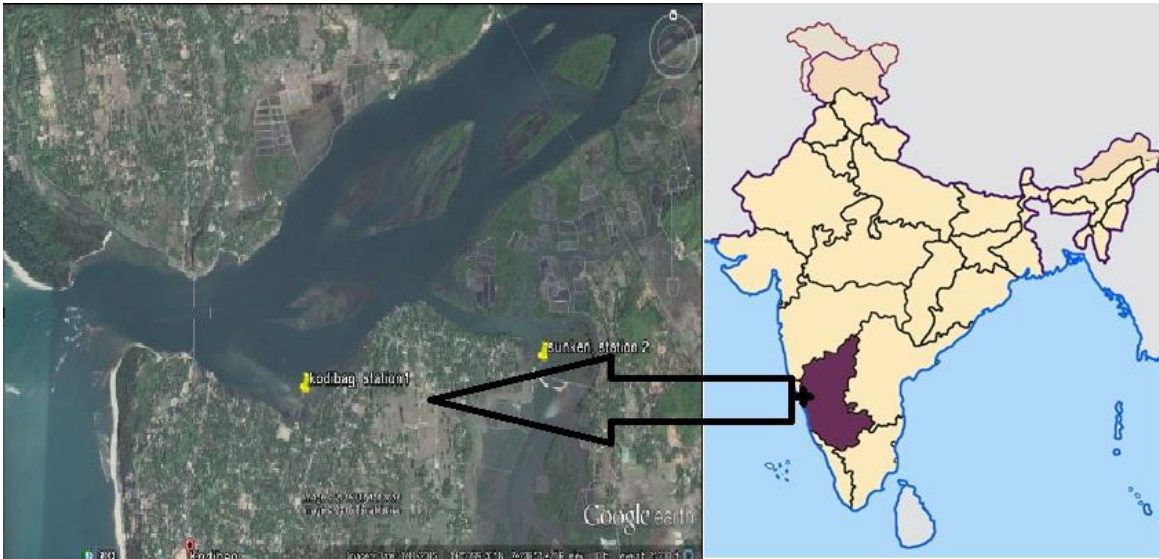


FIGURE 1. Map Showing Kali Estuary Karwar, Karnataka, West Coast of India

Growing population along the bank of estuary and intensive aquaculture together contributing tremendous nutrient discharges into the estuary which is cause of concern for the sea food safety. This study mainly carried out to trace the *V. parahaemolyticus* and its seasonal abundance in the commercial important shellfishes of Kali estuary. Edible shellfishes such as, oysters, clams, shrimps, like *Crassostrea madrasensis*, *Paphia malabarica* and *Fenneropenaeus indicus* (n=10) collected from Kali estuary, preserved in cold condition and taken to the laboratory for the analysis. Before proceeding to the experiment, all the lab equipments were sterilized to avoid contamination. Collected specimens were homogenised with sterilised PBS solution and kept for centrifugation at 2500rpm for 20 min. Thiosulfate-citrate-bile salts sucrose agar (TCBS) is a type of selective agar culture plate that is used in microbiology laboratories to isolate *Vibrio* Sps (Terence and Peter, 1995). TCBS Agar is highly selective for the isolation of *V. parahaemolyticus*. TCBS agar contains high concentrations of Sodium thiosulfate and Sodium citrate to inhibit the growth of Enterobacteriaceae (Dalmin *et al.*, 2002). Suspected colonies

(green, large and smooth) were selected and streaked on to Brain Heart Infusion Agar (BHIA) for further characterization by following biochemical tests outlined in U.S. Food and Drug Administration (FDA) biological manual (1984) along with gram staining. Positive sample of *V. parahaemolyticus* is obtained by Central Marine Fisheries Research Institute (CMFRI), Kochin, Kerala, India.

RESULTS

In the present study the isolation of specific pathogenic bacteria *V. parahaemolyticus* from shell fishes of Kali estuary were made from August 2014 to May 2015. Three highly edible shellfish species were selected for the present study, they are *Crassostrea madrasensis*, *Paphia malabarica* and *Fenneropenaeus indicus*. These species were screened for the presence of *V. parahaemolyticus* by using generally accepted method of isolation on selective specific standard media. All the three species shows presence of *V. parahaemolyticus* in TCBS as well as in the BHIA media. The colonial morphology of *V. parahaemolyticus* on TCBS media is Blue to Green (Figs. 2 - 5).



FIGURE 2: Showing positive sample of *Vibrio parahaemolyticus*



FIGURE 3: Showing the presence of *V. parahaemolyticus* in *P. malabarica*



FIGURE 4: Showing the presence of *V. parahaemolyticus* in *C. madrasensis*



FIGURE 5: Showing the presence of *V. parahaemolyticus* in *F. indicus*.

Presence of *V. parahaemolyticus* is seen in the month of November to May in *P. malabarica*. In *F. indicus* the *V. parahaemolyticus* is seen from January to May study period,

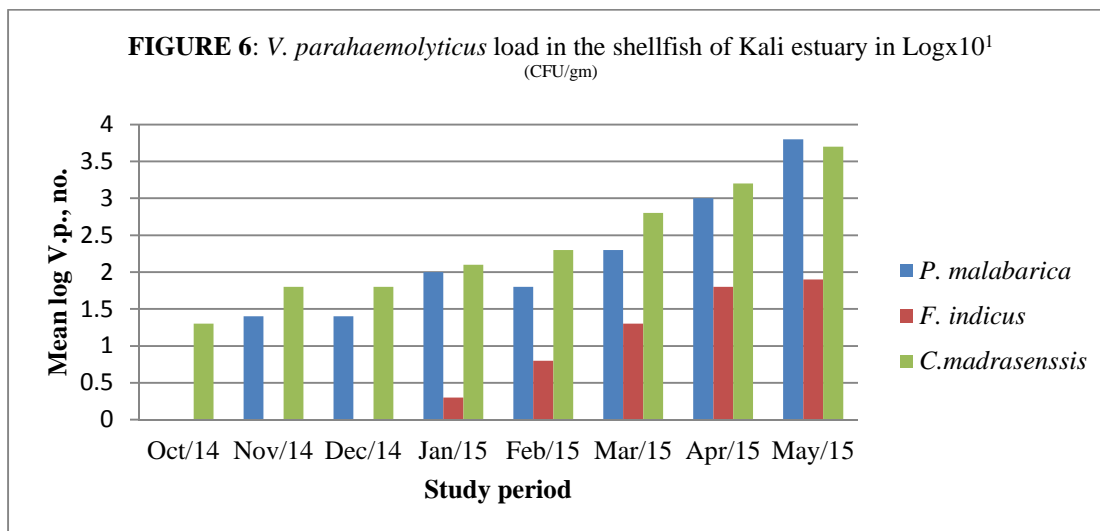
while in *C. madrasensis* its presence was seen throughout the study period (Tab. 1).

TABLE 1: Presence of *V. parahaemolyticus* in the studied specimens of Kali Estuary, Karwar. West Coast of India

Species	August 2014	September	October	November	December	January 2015	February	March	April	May
<i>P. malabarica</i>	-	+	+	+	+	+	+	+	+	+
<i>F. indicus</i>	-	-	-	+	+	+	+	+	+	+
<i>C. madrasensis</i>	+	+	+	+	+	+	+	+	+	+

Vibrio parahaemolyticus being a common inhabitant of coastal water and counts have been found more than 1,300 per gm of oyster (Felsenfeld and Cabarac, 1977) which is very high keeping in mind of public health. In the present study similar tendency was observed and recorded highest number of bacterial count was in *P. malabarica* and *C. madrasensis* (Fig. 6). The presences of *V.*

parahaemolyticus in shrimp *F. indicus* were correlated to the temperature because initial of the study period during the monsoon and winter season bacterial counts were nil and as initiation of summer, colonies' traced till the onset of pre-monsoon period. The presence and dominance of bacterium (*V.p*) recorded among the filter feeders posing threat to the consumer's health.



Indian coastline carved with backwaters and estuaries permits extensive shrimp farming. The intensive expansions of commercial shrimp culture are accompanied by microbial infection (Lightner *et al.*, 1992). Microbes play a key role in shrimp culture either favourably helping in organic degradation and nutrient recycling or detrimental by producing hassle and disease. Species of *Vibrio* particularly

V. parahaemolyticus are among the most common bacterial population associated with penaeid shrimps (Ruangpan and Kitao, 1991). Sindermann (1979) has pointed out that *Vibrios* are the major disease causing bacteria, normally found in the environment and healthy shrimps. In India occurrence of *Vibrio* species in tropical shrimp culture environments is more in the west coast than the east coast

(Shobha *et al.*, 2005) of India. A sudden increase of bacterial load could develop bacterial infection directly and making shrimps susceptible to infection to mass mortality causing heavy economic loss. The microbial contaminated untreated discharges of the intensive culture ponds directly enter into the coastal ecosystem threatening the quality of wild stocks of seafood.

This study documented the presence of *V. parahaemolyticus* in natural shellfishes stocks of the estuary might be indicating that, the anthropogenic and aquaculture discharges which directly entering into the mainstream of estuaries as lack of proper effluent treatment establishments in this region. Even it has been observed in most of the coastal places in India releasing untreated domestic and aquaculture effluents directly into the estuary and seas resulting high prevalence of organic matter. Even though effluent treatments are installed their performance for treating municipal waste water not complying with prescribed standards in state owned plants (Spectrum, 2015). During the summer season with the elevated temperature in association with nutrient influences the bacterial growth leads seafood poisonings. Similar tendencies were also observed in the U.S. and European waters (Annie and Marta, 2012). It is evident from the study that optimum temperature influence growth of *V. parahaemolyticus* among the shellfishes of Kali estuary. Hence, further study is required to trace the presence of *V. parahaemolyticus* and other harmful pathogenic bacteria in the water, sediments and other commercially important edible aquatic organisms of this estuary. Therefore, it is recommended to be away from the consumption of raw or undercooked shellfishes to avoid shellfish poisoning, similarly wounds of human body should not be exposed to contaminated water.

ACKNOWLEDGEMENT:

Our special thanks to Dr. K.K. Phillipose, Scientist In-charge and Dr. S.R. Krupesh Sharma, Principal Scientist, Central Marine Fisheries Research Institute, Research Centre, Karwar, Karnatka for extending their valuable guidance and laboratory facilities to conduct this study.

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