10. SUMMARY

The genus *Vibrio* consists of Gram-negative straight or curved rods, motile by means of a single polar flagellum. Vibrios are capable of both respiratory and fermentative metabolism. In most ways Vibrios are related to enteric bacteria. The Family *Vibrionaceae* is found in the "Facultatively Anaerobic Gram-negative Rods" on the level with the Family *Enterobacteriaceae*. In the recent taxonomy on phylogenetic analysis, *Vibrionaceae*, *Pseudomonadaceae* and *Enterobacteriaceae* are all included in the Gammaproteobacteria. Vibrios are distinguished from enterics by being oxidase-positive and motile by means of polar flagella. Of the Vibrios that are clinically significant to humans, *Vibrio cholerae*, the agent of cholera, is the most important.

Modern knowledge about cholera, however, dates only from the beginning of the 19th century when researchers began to make progress towards a better understanding of the causes of the disease and its appropriate treatment. The 1st pandemic, or global epidemic, started in 1817 from its endemic area in South-East Asia and subsequently spread to other parts of the world.

The emergence of cholera has been a significant public health problem around the world and battle to completely control this deadly disease continues. In addition to human suffering, cholera outbreaks cause panic, disrupt the social and economic structure and can impede development in the affected communities. Prevalence of *V. cholerae* microorganisms in the environment was considered as the most important factor in this regard. Soil, fresh water, sea water, aquatic plants, animals and some birds have been made target for search if they were providing reserve shelter to the causative agent during inter epidemic periods. Multiple environmental factors have
been considered to have the aetiological relationship as no single source is found to host this microorganism in an inter-epidemic period.

The results of environmental sampling and analysis of the environmental and microbiological data have revealed significant correlations of surface temperature, water temperature and rainfall with the occurrence of cholera producing bacteria (presumably *V. cholerae*). The new information on the ecology of *V. cholerae* is proving useful in developing environmental models for the prediction of cholera epidemics.

The results of this present study demonstrate the resource of *V. cholerae* that may prove to be significant in the ecology of epidemic *V. cholerae*. The study encompasses the investigations on the occurrence, distribution and ecology of *V. cholerae*, a human pathogen, implicated in the outbreaks of cholera world over. The following aspects were covered during the present investigation.

The effect of abiotic parameters including air temperature, water temperature, salinity, pH, rainfall, humidity, pressure, dissolved oxygen, calcium, magnesium, phosphate, nitrate and nitrite were monitored over a period of two years (March 2002 to February 2004) at two study areas, the coastal fishing villages, Tharuvaikulam (station 1) and Thirespuram (station 2). Studies were also conducted to assess the variation in densities of the organism in the environment in relation to seasons.

The surface air temperature varied from 26.4 (February 2003) to 33.6°C (June 2002) in station 1. The air temperature in station 2 fluctuated from 26.9 (February 2003) to 33.2°C (April 2002). The water temperature in station 1 fluctuated from 26.2 (December 2003) to 32.1 °C (March 2003), while in station 2, it was between 27.4 (December 2003) and 33.2 °C (October 2002). Significant correlation was noted
between salinity and magnesium in station 1 during monsoon 2002 and nitrate during monsoon 2003. There was significant correlation between pH and calcium during summer 2002, and with magnesium during monsoon 2002, premonsoon 2003 and postmonsoon 2004. There was significant correlation between pH and nitrate, nitrite, dissolved oxygen during postmonsoon 2003 and phosphate, during summer 2003 in station 2. Two way ANOVA test confirmed the prevalence of highly significant (P<0.05) variation among different seasons and not stations.

Summer seasons showed maximum pressure and the rest of the seasons with no marked changes. Significant correlation was observed in station 2 between water temperature and calcium during summer 2003. The maximum level of magnesium content was recorded during summer followed by premonsoon, postmonsoon and monsoon seasons. The concentration of nitrate in station 1 fluctuated between 0.105 in January 2004 and 1.04 µg l⁻¹ in May 2002. Postmonsoon season alone showed the maximum level in nitrite. The phosphate concentration ranged between 0.195 (August 2002) and 4.6 µg l⁻¹ (November 2003) in station 1, while in station 2, it ranges from 0.505 (March 2002) to 6.47 µg l⁻¹ (September 2002). During summer 2002, surface temperature showed significant correlation between magnesium and dissolved oxygen in station 2.

The occurrence of outbreaks of cholera, mainly in coastal communities in Africa in 1970 and in Latin America in 1991, and the appearance of the new serotype *V. cholerae* O139 in India and subsequently in Bangladesh have stimulated efforts to understand environmental factors influencing the growth and geographic distribution of epidemic *V. cholerae* serotypes. Because of the severity of recent epidemics,
cholera is now being considered by some infectious disease investigators as a "reemerging" disease, prompting new work on the ecology of Vibrios.

The total heterotrophic bacterial population was almost similar in all seasons. There was no significant seasonal variation on the total heterotrophic bacterial populations present in water, but station wise significance (P<0.05) was recorded. The faecal coliforms population was found to be the maximum in station 1 during post monsoon seasons of 2003, whereas in station 2, the faecal coliforms count in water sample fluctuated from 2.57 MPN/100ml in March 2003 to 8.87 MPN/100ml in September 2003 and January 2004. Analysis of variance showed no significant differences in the distribution of fecal coliforms in water between the seasons and stations. The faecal coliforms counts in station 1, increased from 2.30 MPN/100ml in September 2003 to 8.87 MPN/100ml in April, June 2002, March 2003 and February 2004, while in station 2, the faecal coliforms counts were higher during summer season followed by monsoon, post monsoon and pre monsoon.

Total heterotrophic bacteria in Sardinella longiceps at station 1, fluctuated between 2.18 (July 2003) and 2.45 log (March 2002). The maximum fecal coliforms were recorded during the pre monsoon period in 2003, followed by postmonsoon and summer seasons. Analysis of variance showed no significant differences in the distribution of V. cholerae between the seasons and among stations. The counts for faecal coliforms in Lethrinus nebulosus increased from 2.63 MPN/100ml in March 2002 to 15.33 MPN/100ml in June 2002 in station 1. The faecal coliforms population was found to be the maximum during summer seasons in 2002, whereas in station 2, the FC count fluctuated from 2.20 MPN/100ml in January 2003 to 8.87 MPN/100ml in June and November 2003. The maximum V. cholerae was recorded during the
summer period of 2003 from *Lutjanus ruselli*. Analysis of variance showed significant differences in the distribution of *V. cholerae* between the seasons (P<0.05) and among stations (P<0.05). Total heterotrophic bacteria in *Portunus pelagicus* at station 1, fluctuated between 2.04 (August 2002) and 2.28 log (January 2004). The total heterotrophic bacterial population was almost similar in all the seasons. In station 2, the total heterotrophic bacteria fluctuated between 2.04 (July 2002) and 2.29 log (December 2002). There was no significant seasonal variation on the total heterotrophic bacterial populations. Samples collected during summer in 2002 contained high number of *V. cholerae* level. The counts for faecal coliforms in *Portunus sanguinolentus* increased from 2.30 MPN/100ml in March 2002 to 8.87 MPN/100ml in August and September 2003 in station 1. The faecal coliforms population was found to be the maximum during premonsoon season in 2003, while in station 2, the FC count in *Portunus sanguinolentus* fluctuated from 2.50 MPN/100ml in May 2003 to 8.87 MPN/100ml in August and December 2003.

The importance of good environmental conditions and management practices in preventing outbreaks of diseases is well understood. Although in recent times, significant progress has been made in vaccine development, a fully effective product is yet to be made commercially available. Antimicrobial chemotherapy is still essential in controlling epizootics and is likely to remain so in the immediate future. The prevalence of drug-resistant plasmids (R plasmids) in the Enterobacteriaceae and related genera of bacteria has become a problem of prime importance in chemotherapy, live stock farming and fish breeding. Multiple antibiotic resistance in bacterial pathogens is now a common phenomenon in developing countries. In this study, the patterns of resistance to 10 antibiotics by *V. cholerae* species isolated from
both environmental and seafood samples were investigated. The potential impact of antibiotic resistance in relation to health risk is discussed. The frequency of resistance to the antimicrobial agents such as ampicillin, chloramphenicol, bacitracin, erythromycin, gentamycin, streptomycin, oxytetracycline, vancomycin, penicillin and neomycin was 88, 46, 8, 64, 13, 85, 18, 21, 84 and 18% respectively. More than 50% of the strains were resistant to ampicillin, chloramphenicol, erythromycin, penicillin and streptomycin. Maximum sensitivity was shown towards gentamycin, while bacitracin, neomycin, oxytetracycline and vancomycin were also sensitive. About 10-20% of the studied strains showed a 3-5 multiple antibiotic resistance (MAR) pattern. Only small percentage of the studied strains showed a 0-2 and 6-9 MAR.

The sediments seem to play a significant buffering role for the survival of *V. cholerae* by offering protection to them when environmental conditions are not conducive. Texture, an important grain size attribute, provides useful information on mode and extent of transport and environment of deposition of clastics. In this context, the study was conducted to understand the texture and nature of transportation and deposition of the sediments in the study area, which might influence the bacterial incidence due to its nutrient cycling.

The mean grain size of samples in station 1 varied between 1.80 and 2.88 \( \phi \) and for station 2, it ranged between 1.65 and 2.90\( \phi \) indicating that the sediments are mostly medium to fine size in Wentworth’s size class. The graphic kurtosis values for samples at station 2 ranged from 0.35 to 2.46\( \phi \) falling in very platykurtic to very leptokurtic types. For samples at station 1, the values varied between 0.26 and 3.47\( \phi \) (very platykurtic to extremely leptokurtic). Grain size studies were carried out in the collected samples for a period of two years. Grain size was plotted on the horizontal
axis in arithmetic scale and cumulative weight percentage was plotted on vertical axis in probability scale running from 0 to 100%. It is vividly concluded that the samples fall mainly in the field of medium sand to fine sand, which indicates a moderate wave action along the shoreline of the study areas.

Different national and international pharmaceutical companies are utilizing plant based formulations in treatment of various diseases and disorders worldwide. Preparations from plants were the original therapeutic interventions used by man to control diseases in humans and livestock. Nearly all cultures from ancient times to the present day have used plants as a source of medicines. The World Health Organization has estimated that more than 80% of the world’s population in developing countries depends primarily on herbal medicine for basic healthcare needs.

Aqueous extracts of different aerial parts (leaves, flowers, seeds, fruits, barks) and subterranean parts (roots, rhizomes) of the plants were investigated. Among the 45 medicinal herbs, only 30% (13 plants) of the medicinal herbal decoctions tested exhibited a pronounced antibacterial effect against *V. cholerae*. Antibacterial activity was found in 13 decoctions of different plant parts. Significant reduction in the population of *V. cholerae* occurred when the *C. ramosus* meat was dipped into the potent herbal decoctions. All these herbal decoctions inhibited completely the growth of *V. cholerae* on *C. ramosus* meat at 96th hour. However all the thirteen herbal decoctions experimented exhibited some reduction in the *V. cholerae* load at 48th hour.
The information available to date provided fascinating insight into the ecology, systematics, epidemiology and pathogenicity of *V. cholerae*. From the studies of *V. cholerae* in stations 1 and 2 and also from studies made in other environments, it appears that the findings and conclusions arrived are accurate and that the organism is indeed an autochthonous species in Tuticorin coastal environment. The salinity, pH, temperature and nutrient levels in Tuticorin coastal waters provide a very suitable environment for *V. cholerae*. The fact that this coast offers an ideal environment for *V. cholerae* that is reflected in the abundance of the organism and the frequency of isolation. The warm, nutrient rich waters of Tuticorin are excellent habitats for *V. cholerae*. The presence of bacterial emerging pathogen *V. cholerae*, in the coastal waters of Tuticorin, India, as well as the role played by seafoods in the spread and persistence of this bacterium, is of great importance from both epidemiological and ecological points of view. Since this organism is distributed in aquatic environments, it is essential to gain a more detailed knowledge about the ecology of this organism. Unless the ecology and ecotoxicology of this bacterium is fully understood and addressed, the control and prevention of infections caused by these pathogens may not be possible.

The high incidence of these pathogens in commercially important species of fish and shellfish is to be noted with concern from public health point of view, since most outbreak of *V. cholerae* are attributable to consumption of contaminated seafood. It seems virtually impossible to protect raw seafood from such defilement, but with proper care in handling and processing, the *V. cholerae* problem can be practically controlled to a very large extent. Education level also plays an important role by the knowledge of creating awareness among the local fisher folk. It has been
shown that the more education they have, the more they know about the way to take care of themselves with diarrhoeal diseases caused by enteric pathogens. The most important means of controlling human infection lies in simple hygienic measures aimed at preventing multiplication of the vibrios in seafood.