CHAPTER 8

Conclusion and Recommendation
8.1. Summary and conclusion

Several studies have confirmed that water-related diseases not only remain a leading cause of morbidity and mortality worldwide, but that the spectrum of disease is expanding and the incidence of many water-related microbial diseases is increasing. Recent studies have shown links between pathogen and changing ocean conditions, including human diseases such as cholera. *Vibrio cholerae* is a well-known human pathogen that has caused cholerae epidemics worldwide and continues to be prevalent in many developing countries. In spite of numerous studies over more than a century, the epidemiology and ecology of cholera remain mysterious and challenging and several gaps remain in understanding *V. cholerae* dynamics in the environment. Specific objectives of the study were: (1) to identify the influence of environmental factors (biotic and abiotic) on *V. cholerae* dynamics and seasonality (2) to assess the distributional status of *V. cholerae* and other potentially pathogenic enteric bacteria along the Veli-Kochi coastal tract (Southern Kerala, India) and (3) to evaluate the hydrographical regime and pollution status of the above study area.

Based on the objectives, seasonal samples were collected from four coastal locations of southern Kerala, viz. Veli, Neendakara, Alleppey and Kochi, each with 5 sampling sites i.e., 0km (nearshore/estuary), 1km, 3km, 5km and 10km respectively from the shore/estuary for a period of one year during the cruises of *Sagar Purvi* and *Sagar Paschimi*, the coastal research vessels of Ministry of Earth Science (MoES), Govt. of India and it was exposed to analytical techniques such as the physico-chemical, marine biological and microbiological parameters of water. The parameters for sediment analysis included percent composition of sand, silt, clay and organic carbon concentration and *V. cholerae* population in both sediment and benthic
organisms. Four major benthos phyla were selected for the evaluation of *V. cholerae* association with benthic organisms, Protozoa (foraminifera), Annelida (archiannelida and polychaeta), Arthropoda (decapoda and copepda) and Mollusca (gastropoda and pelecypoda). Physico-chemical analysis of water studied were water temperature, salinity, total suspended solids, pH, dissolved oxygen, biological oxygen demand, nitrite, nitrate, ammonia, total nitrogen, inorganic phosphate, total phosphorous and silicate. Biological analysis of water includes the distribution of different pathogenic enteric bacteria (total coliforms, faecal coliforms, *Faecal streptococci, Shigella* spp, *Salmonella* spp, *V. parahaemolyticus* and *V. cholerae*) and different phytoplankton and zooplankton taxa.

Microbiological studies determined that an area extended for more than 3km seaward at least had been severely affected by the pathogenic enteric bacteria as well as faecal indicator bacteria and there were hardly any samples throughout that had counts of bacteria that would be considered safe. Estuaries were found to be much polluted than coastal sites, as riverine discharges which have been ascribed as stimulants for higher occurrence of microbial population contribute heavy bacterial load. Especially Kochi estuary with the perpetual presence of *Shigella* spp, *V. parahaemolyticus* and *V. cholerae* suggests a potential for disease outbreak as their infectivity is growth rate dependent. Throughout the study, monsoon season reported to have the high incidence of all indicator bacteria except *V. cholerae*, anticipated as the heavy monsoon showers bring in heavy land run-off, resulting increased level of waste discharge into the system. Also the inactivating effect of major self-purifying factor such as sunlight is very much reduced during the monsoon months as the sky is almost always overcast. Among the indicator bacteria studied, *V. cholerae* found to be rather stable and had higher survivability rate with percentage incidence of higher
than the traditional indicator bacteria (TC, FC and FS). *V. cholerae* also showed significant relationship with all the traditional indicator bacteria, suggests that both quantitatively and qualitatively abundance of *V. cholerae* can determine faecal pollution, could be used as a faecal pollution indicator bacterium, especially in the marine environment where traditional indicator bacteria fail to survive.

Study does not try to elucidate the fate of different indicator as well as pathogenic bacteria in the marine environment. However it does demonstrate how environmental factors contribute cholera seasonality, *V. cholerae* survival during the seasonal shifts and the natural reservoirs that may prove to be significant in the ecology of *V. cholerae*. In the pelagic environment, the prevalence of pathogenic *V. cholerae* appears to be influenced by the physico-chemical features of the environment and demonstrated a spatiotemporal correspondence with regard to environmental variations. Temperature, salinity and nutrient concentration are the major factors affecting the distribution and relative abundance of *V. cholerae*. Multiple linear regression model suggest the variable with the greatest explanatory power was temperature which explained 34.9% of the variation in *V. cholerae* data followed by total phosphorous of 24.7%. Kochi. Post monsoon reported to have the higher incidence of *V. cholerae* followed by premonsoon. *V. cholerae* showed significant positive relationship with water temperature during monsoon and during non-monsoon, relative abundance in *V. cholerae* was found with concomitant increase in nutrient concentration. Throughout the study Kochi reported to have the mean high nutrient concentration and *V. cholerae* population and Veli the least of them all. Study also suggests the development of a nutrient management strategy is essential component in Kochi, otherwise it adversely affect the water quality as well.
In order to better exemplify the *V. cholerae* – biotic association (phytoplankton, zooplankton and benthic organism) and the role of environmental factors on such association, two diverse marine environments which better reflect a gradient in the hydrographic and sedimentological regime were selected, Veli and Kochi, former with low nutrient loading and latter high nutrient loading. In the pelagic environment, study observed direct relationship between *V. cholerae* and plankton (both phytoplankton and zooplankton) with respect to the relative decrease in nutrients and temperature and abrupt shift in salinity in the environment, suggest that plankton might be associated with this pathogen during unfavorable condition. This association perhaps is the major reason behind *V. cholerae* seasonality, such that during monsoon a strong association of *V. cholerae* with biotic organisms and during post-monsoon they remain in free form. Interestingly, *V. cholerae* populations in plankton fraction were also found to be high during monsoon. The mean *V. cholerae* concentrations in the water fraction (230CFU ml⁻¹) was up to 5 and 6 orders of magnitude less than the mean concentration *V. cholerae* in phytoplankton fraction (1.49 x 10⁶ CFU g⁻¹) and zooplankton fraction (1.07 x 10⁷ CFU g⁻¹) respectively. Among the zooplankton studied crustacean copepods showed close association with *V. cholerae* population. Copepods in the benthic environment also showed the mean high abundance of *V. cholerae* (23.41 x 10³ CFU g⁻¹) than sediments (16.3 x 10³ and 19 x 10² CFU g⁻¹ in Kochi and Veli respectively). *Vibrio cholerae* was also detected in archiannelida, polychaeta, foraminifera, gastropoda and pelecypoda, all new sites for this bacterium. Generally, abundance of *V. cholerae* in copepods was observed with increased sand concentration. Seasonal salinity variability and temperature were also found to influence *V. cholerae* benthic attachment.
Overall study suggests that zooplankton were the largest reservoir of *V. cholerae*. Higher incidence of *V. cholerae* with copepods indicting the chitinous structures acts as carbon source during nutrient reduced condition and confirm the ability of chitinous structures to concentrate and to retain bacteria (recorders of present or even past microbiological pollution). Study also suggests that under high nutrient loading routine monitoring of water quality is agreeable, but with low nutrient and temperature conditions phytoplankton groups especially *Chaetoceros*, *Rhizosolenia* and *Skeletonema* and zooplankton taxa especially crustacean copepod should be used as a reservoir and also as an indicator for the presence of *V. cholerae*. However, in the benthic environment, especially in areas with high sand concentrations and low temperature and salinity regime, decapoda and copepoda should be used as a preferential tool to estimate the abundance of *V. cholerae*. The association of *V. cholerae* with a wide diversity of biotic organisms is a new and important finding of the present study, indicating that zooplankton, phytoplankton and benthic organism is an important and still underestimated reservoir of these bacteria. The new information on the ecology of *V. cholerae* is useful in developing environmental models for the prediction of cholera epidemics.
8.2. Recommendation for Future Research

There is a great need to improve environmental monitoring and surveillance systems in low- and middle-income countries such as India. New research initiatives should focus on collecting high-quality, long-term data on climate-related health outcomes with the dual purpose of understanding current climate-health associations and predicting future scenarios. Research on the effects of climate variation on infectious disease incidence and geographic range in these diverse contexts is providing the basis for developing climate-based early warning systems for disease risk. Such studies also represent a necessary first step toward anticipating how climate change may alter infectious disease dynamics in various ecological frameworks. So research on the links between climate and infectious diseases must be strengthened in order to examine the consistency of climate/disease relationships in different social contexts and across a variety of temporal and spatial scales. Clarifying the causal pathways linking climate to disease prevalence will require additional knowledge of the ecology of the pathogen and the transmission dynamics of infectious disease. Therefore, it is important to develop a comprehensive catalog of climate change and associated health outcomes across the range of environments and populations likely to be affected. A better understanding of the effects of climate change on health in India will be best achieved through studies specific to climates and populations in India.

Accordingly, improved methodologies and assessments are needed to better understand the dynamics of bacterial community change. The technique, called the Polymerase Chain Reaction (PCR), is now widely used in medical, forensic and environmental laboratories. Not only does the method allow investigators to
discriminate between micro-organisms from different sources, it can be used to
detect extremely small quantities of the nucleic acid: equivalent to a single micro-
organism. Other recently-developed technologies are being assessed for their
application in water microbiology. Flow cytometry is a powerful technique using
laser light to quantify particles or to recognize structural features of cells. By
measuring the scatter and wavelength of light as a particle intercepts the beam,
information can be gained that allows the rapid quantification of the organisms. The
analytical capability of the technique can be further enhanced by use of fluorescent
monoclonal antibodies that are specific for a particular pathogen.

The ability of \( V. \) cholerae to form biofilms may enable it to aggregate more
effectively in the aquatic environment, as suggested in a study of \( V. \) cholerae O139
during an outbreak in Calcutta, where \( V. \) cholerae O139 was found to be more
abundant in surface waters than in sediment or on plankton (Ghosh et al., 1994).
Ecological investigation focusing on such studies should be performed in different
condition so as to reach accurate precisions. The discovery that cholera is transmitted
by plankton is an important one which may be the basic assumption for the
prediction of cholera epidemics in the future also, correlating the population
dynamics between \( V. \) cholerae and planktons. However, which plankton species act
as the major reservoir of this bacterium is yet to be far predicted. There is a
commensal relationship—which may prove to be symbiosis—between \( Vibrio \)
bacteria and plankton. It is not enough to say that Vibrio cholerae growth correlates
with sea surface temperature and salinity; it is important to recognize the ecological
interactions that produce these correlations. Research in this area must be both tem-
porally and spatially specific. Furthermore, it requires local monitoring of the
appropriate climate and disease variables (Patz et al. 2002) because underreporting
impedes the development of effective prevention strategies. It is critical to build a data infrastructure and conduct such research in India so that region-specific models based on climate and health can be developed. A systems approach focusing on health outcomes is critical to the success of future research in this area (Batterman et al. 2009). As prediction models evolve, region-specific action plans and adaptation strategies can be developed. A more complete and clearer understanding of the ecology of *V. cholerae* is critical to identify and comprehend the seasonality and regional mechanisms as a function of environmental factors for the prediction and management of this disease. The sooner these consequences are estimated and communicated the better will be the chance of averting future retrograde policy decisions.

Considerable effort has been devoted to finding an effective vaccine. Because of the oral–fecal transmission route, recent vaccines have been oral, based on inactivated bacteria or live attenuated strains. Despite inducing seroconversion, the low level of protection could not justify their use. Nevertheless, some live attenuated strain vaccines, such as Peru-15, are still being developed and tested (Ryan, 2006).

For these new surveillance methods and analytical techniques to be effective, countries like India will need to enhance their human and technical capacity for risk communication. For cholera prevention knowledge to be translated into preventive health behaviours. This could take the form of public education on climate change and associated health impacts to enhance awareness and to influence lifestyle, behavior, and individual choices to protect and improve health. Such health promotion materials could manifest as low-tech flyers and advertisements as well as more high-tech materials including web-based and mobile-phone–based alerts. Furthermore, outbreak investigations do not usually focus on whether the population
is adopting the recommended disease prevention behaviours, which is particularly important for preventing recurrences of outbreaks. Knowledge, attitudes, and practices studies can provide this information and can suggest potential barriers to the adoption of disease control measures, and be used to refine and focus health education interventions.