INTRODUCTION
1. INTRODUCTION

Traditionally, seafood is a popular food diet in Indian sub-continent and other parts of the world. In India, particularly the coastal areas, seafood provides the main source of dietary animal protein and also generates income avenues for 14 million fisher folk and people associated with seafood industry. Seafood sector is playing an important role in the economy and nutritional security of the nation. The export earnings from seafood for India in the year 2007-08 were to the tune of over Rs. 7620 crores (Anon., 2008). Today, more people are turning towards fish as a healthy food due to low fat content and presence of n-3 polyunsaturated fatty acids in fish. However, consumption of fish and shellfish may also cause various diseases to the consumers due to infection or intoxication by food-borne pathogens. The presence of food borne pathogens also cause huge monetary loses to the fisherman and the exporters. The seafood exporters in the country have been facing tremendous challenges in meeting the food safety requirements from the European Union (EU) and United States. The EU commission has imposed border testing of frozen seafood products for *Salmonella* and *Vibrio* spp. which resulted in a decline in export to the EU countries.

Seafood being a relatively high risk perishable food, are subjected to a range of food safety requirements related to general biological and chemicals hazards. Among foodborne pathogens, *Salmonella* comes top in the rank for being responsible in foodborne outbreaks. Food borne pathogens are inherent in seafood from aquatic and terrestrial environments. In a 2-year period (1980-1981) 8.7% of disease outbreaks in the Netherlands were associated with seafood and 10.1% of outbreaks in the United States during a period of 1972-87 were connected with seafood (Huss et al., 2000). The
loss due to food borne outbreaks costs the United States, alone $1.1 billion to 1.5 billion annually. Food borne outbreaks are not properly documented in developing countries, unlike the western counterparts; hence, less number of reports are available in these countries. Presence of *Salmonella* in seafood is well documented. In numerous incidences, *Salmonella* serovars have been isolated from seafood in India and abroad.

*Salmonella* is a leading food borne pathogen; causes both typhoid fever and salmonellosis illnesses in humans. Till date, more than 2540 *Salmonella* serotypes have been identified, based on somatic (O), flagellar (H) and capsular (Vi) antigenic profile (Popoff et al., 2004). The natural habitat of *Salmonella* spp. is in the gastrointestinal tract of animals, birds, reptiles and even some serotypes have been isolated from marine sources. Outbreaks due to *Salmonella* have been associated with consumption of chilled boiled salmon, halibut, cooked cockles, fish and chip (Francis et al., 1989). The incidences of *Salmonella* in India associated with seafoods were reported in some of the earlier studies (Iyer and Shrivastava, 1989b; Nambiar and Iyer, 1991; Hatha and Lakshmanaperumalsamy, 1997; Shabarinath et al., 2007).

Most commonly, conventional culture method has been used for the isolation and identification of *Salmonella* serotypes in seafood. The basic principle behind the isolation and identification of *Salmonella* in culture method is the biochemical substrate utilization pattern, although, considerable variations observed in biotyping pattern. Majority of *Salmonella* are recognized as non-lactose fermenters (lac^-) and hydrogen sulfide (H2S^+), although, majority of *Salmonella enterica* subsp. *arizonae* and *Salmonella enterica* subsp. *diarizonae* are lactose fermenters and certain H2S negative *Salmonella* serovars are also available. The conventional approach requires confirmatory test of all typical and atypical colonies on selective plates and it becomes
very cumbersome to identify these suspected Salmonella isolates. Hence, alternative molecular approaches need to be incorporated in the detection assay. The process of isolation and identification of Salmonella in seafood by conventional method requires multiple steps of pre-enrichment, selective enrichment, followed by plating on selective media and finally biochemical confirmation with key reactions. The entire process takes 5-7 days to identify a Salmonella isolate. Thus, there are considerable interests in the development of more rapid techniques, particularly for detection of Salmonella in seafood. Different array of tests have been developed in the form of miniaturized biochemical kits, immunoassays and DNA-based tests for rapid screening of large number of food or seafood in a short duration. Rapid methods provide an alternative approach for screening large number of samples in a short duration. A large number of modern rapid methods have been approved by AOAC and other international agencies such as USDA and NMKL (Swaminathan and Feng, 1994; Fung, 1997). The main disadvantage of the commercial kits available in market is that they are expensive in nature. Thus, development of indigenous rapid, sensitive and competitive technique based on PCR and DNA probe assays for identification of Salmonella serovars in seafood would be an ideal step for rapid screening of seafood samples.

In epidemiological studies, biotyping, serotyping, and antimicrobial typing methods have been frequently used for characterization of Salmonella serotypes from different environments. Biotyping assay consists of the utilization pattern of various sugars, amino acids and other organic compounds and is most simple and commonly used typing technique. Disadvantage of this method is that it is less discriminating, in nature, between strains. Serotyping is another phenotypic method, which confirms the relatedness among the isolates from common and different environments based on antigenic property. This technique is quite specific and most commonly used for
characterization of Salmonella isolates but it is complex and laborious in nature. Indiscriminate use of antibiotics in humans and farm animals has led to development of antibiotics resistance in bacterial pathogens. Use of antibiotics in the aquaculture ponds also contributed to development of antibiotics resistance in bacteria. Antimicrobial resistance typing profile gives the impact of chemical hazards on environment, particularly in microbes. This technique has been successfully used for the detection of antimicrobial resistance profile of Salmonella serotypes. The microbial typing methods have been used in wide range of microorganisms, but none of these typing methods offers an ideal approach for the subtyping of microbial species. Thus, the combination of different methods may be the best approach to characterize the Salmonella isolates.

The dynamics of species variability arise from bacterial mutation and conjugative intra and inter generic exchange of transposons and plasmids encoding determinant traits. Different molecular typing methods based on the variation in genetic makeup have been now used in complement with traditional typing methods for fingerprinting of Salmonella serotypes. Nucleic acid, protein and lipopolysaccharides are the only macromolecules that carry information in their sequences and compositions to allow the study of microbial diversity and the development of molecular typing methods that would be the more holistic approach for characterization of Salmonella isolates. Molecular typing of a Salmonella serotypes can be based on plasmid typing, enterobacterial repetitive intergenic consensus sequences (ERIC)-PCR, virulence gene characterization, and pulsed field gel electrophoresis (PFGE) analysis. These molecular fingerprinting methods will provide the genetic variation in Salmonella serovars associated with seafood in this part of the country.

Against this background, the main objectives of the proposed investigations are:
• Isolation and characterization of Salmonella serovars from fresh and unprocessed seafood from Cochin (India).

• Development of biotyping profile of different serovars based on utilization of various sugars and amino acids.

• Antibiotic resistance profile of Salmonella serovars isolated from seafood.

• Development of molecular typing patterns based on PCR-ribotyping, for Salmonella serovars associated with seafood.

• PFGE based fingerprinting profile of Salmonella serovars.

• Characterization of different Salmonella virulence genes.

• Development of rapid and sensitive detection assays for Salmonella in seafood.

• Quantitative detection of Salmonella in seafood by real-time PCR.

About this thesis

The present investigation was envisaged to determine the prevalence and identify the different Salmonella serovar in seafood from Cochin area. Though, the distribution of Salmonella serovars in different seafood samples of Cochin has been well documented, the present attempt was made to identify the different Salmonella serovars and determine its prevalence in various seafoods. First part of this investigation involved the isolation and identification of Salmonella strains with the help of different conventional culture methods. The identified isolates were used for the further investigation i.e. serotyping, this provides the information about the prevalent serovars in seafood. The prevalent Salmonella strains have been further characterized
based on the utilization of different sugars and amino acids, to identify the different biovar of a serovar.

A major research gap was observed in molecular characterization of *Salmonella* in seafood. Though, previous investigations reported the large number of *Salmonella* serovars from food sources in India, yet, very few work has been reported regarding genetic characterization of *Salmonella* serovars associated with food. Second part of this thesis deals with different molecular fingerprint profiles of the *Salmonella* serovars from seafood. Various molecular typing methods such as plasmid profiling, characterization of virulence genes, PFGE, PCR- ribotyping, and ERIC–PCR have been used for the genetic characterization of *Salmonella* serovars.

The conventional culture methods are mainly used for the identification of *Salmonella* in seafood and most of the investigations from India and abroad showed the usage of culture method for detection of *Salmonella* in seafood. Hence, development of indigenous, rapid molecular method is most desirable for screening of *Salmonella* in large number of seafood samples at a shorter time period. Final part of this study attempted to develop alternative, rapid molecular detection method for the detection of *Salmonella* in seafood. Rapid eight–hour PCR assay has been developed for detection of *Salmonella* in seafood. The performance of three different methods viz., culture, ELISA and PCR assays were evaluated for detection of *Salmonella* in seafood and the results were statistically analyzed. Presence of *Salmonella* cells in food and environmental has been reported low in number, hence, more sensitive method for enumeration of *Salmonella* in food sample need to be developed. A quantitative real-time PCR has been developed for detection of *Salmonella* in seafood. This method would be useful for quantitative detection of *Salmonella* in seafood.
The thesis is divided into five major chapters and each chapter is further divided into subheads. The first chapter highlights the identification of problem and the theme of research work with suitable objectives. Second chapter deals about the review of literature. The review includes taxonomical status, morphology, isolation, growth and biochemical characteristics and antibiotics resistance of Salmonella. Different method of isolation and identification of Salmonella in food has been reviewed and more attention is given to rapid, immunological and molecular methods. Different typing methods such as biotyping and serotyping of Salmonella spp. are also reviewed. The epidemiology of salmonellosis and its public health significance and final part the review of literature covered the distribution of Salmonella in seafood, national and international perspectives. A brief review of statistical analysis is also included in the review of literature. Third chapter deals with material and methods. All method employed in the investigation are presented in detail. In chapter 4, results and discussion are presented. Results are mostly in tables and figures and also presented in dendrograms formats. The findings are discussed in detail. Finally, a summery of the entire work is presented in the chapter 5 and a detailed bibliography of the all citation made in the thesis is shown at the end of the thesis. A list of the publication from the study is also appended at the end of this thesis.