1. INTRODUCTION

Contamination of seafood with *Salmonella* is a major public health concern. The natural habitat of *Salmonella* is the gastrointestinal tract of animals, including birds and man (Pelzer, 1989) and finds its way into the river water, coastal and estuarine sediments through fecal contamination. Aquatic environments are the major reservoirs of *Salmonella* and aid its transmission between the hosts (Foltz, 1969; Cherry *et al.*, 1972). The survival rate of *Salmonella* in such aquatic environments is very high, outliving even *Vibrio cholerae* in highly eutrophic river water (Chao *et al.*, 1987; DiRita, 2001). They also survive the sudden increase of salinity and the consequent osmotic stress, which follows the mixing of sewage effluent and brackish water (Mezrioui *et al.*, 1995). Moreover, filter-feeding organisms such as oysters, shrimps (Martinez-Urtaza *et al.*, 2003) and shellfish concentrate all particulate matters in water including pathogenic bacteria and this might explain the presence of *Salmonellae* in seafood. In shrimp processing industry, the principal sources of *Salmonella* contamination are cultivated ponds and coastal water used for handling and processing the seafood, along with rodent and lizard droppings (Kaura and Singh, 1968; Gopalakrishnan and Verma, 1990). Many factors including inadequate supplies of clean water, inadequate sanitary measures, lack of food hygiene and food safety measures have been responsible for increased incidence of food-borne salmonellosis. In India, a number of investigators have reported *Salmonella* in seafood (Gopalakrishnan and Joseph, 1980; Gopalakrishnan and Damle, 1981; James *et al.*, 1985). Hatha and Lakshmanaperumalsamy (1997) reported that 14.25% of the fish samples and 17.39% of crustacean samples that were analyzed were found to be contaminated with *Salmonella*. An earlier report (Iyer and Shrivastava, 1989b) noted the presence of *Salmonella* in 12% peeled and deveined shrimp, 10% headless shell-on shrimp, 14% peeled undeveined shrimp, 25% catfish and 20% seer fish.

Most of the earlier studies on prevalence of *Salmonella* in tropical seafood were conducted using conventional culture methods, which are time consuming. During recent years, molecular techniques are being increasingly used for detection of pathogens in foods. The importance of PCR technique for the detection of all species of *Salmonella* has been well described (Lin and Tsen, 1996; Brasher *et al.*, 1998). The use of PCR based detection of *Salmonella* in oysters has also been documented (Bej *et al.*, 1994; Vantarakis *et al.*, 2000).

However, variations in limits of detection and accuracy have been observed with the different primers (Aabo *et al.*, 1993; Baumler *et al.*, 1997; Malorny *et al.*, 2003). With this background, the objective of the present study was to evaluate the efficiency of three different primer pairs viz., *hns* (Jones *et al.*, 1993) which targets a gene encoding a DNA binding protein, *invA* (Rahn *et al.*, 1992) and *invE* (Stone *et al.*, 1994) targeting two genes in
the inv locus that transcribes putative invasion proteins, for the detection of seafood-associated Salmonella.

The role of molecular subtyping to elucidate the genomic diversity between the different Salmonella isolates is well documented (Reeves et al., 1989; Williams et al., 1990; Welsh and McClelland, 1991) and this would yield valuable information in epidemiology and in tracking the source of contamination. Recently, many DNA based techniques have been developed to compare and differentiate pathogenic bacteria including Salmonella. Earlier reports show that RAPD-PCR, which uses single primer of arbitrary nucleotide sequence, can be successfully applied for molecular fingerprinting. These primers are accessible to random segments of genomic DNA to reveal polymorphisms (Williams et al., 1990; Welsh and McClelland, 1991). The resulting fingerprints can be of epidemiological value and RAPD can be a powerful tool to assess genetic diversity in situations where traditional typing methods like Multilocus Enzyme Electrophoresis (MLEE) and ribotyping could not discriminate the different strains (Tenover et al., 1995). The usefulness of ERIC-PCR (Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction) in the genetic discrimination of different Salmonella serotypes has been described (Millemann et al., 1996; Lim et al., 2005). Lim et al. (2005) have even described the usefulness of a composite analysis of two typing methods viz., RAPD and ERIC-PCR in discriminating Salmonella. Hence, we used RAPD and ERIC-PCR to discriminate the different seafood isolates of Salmonella based on their genetic diversity. To our knowledge, ours is one of the first attempts to assess the genotypic variation in seafood-associated Salmonella Weltevreden which is the predominant serovar in South East Asia.

The virulence of some Salmonella serovars is host specific. S. enterica serovar Typhi causes Typhoid fever in humans but not in mice (Carter and Collins, 1974). On the other hand, S. enterica serovar Typhimurium possesses a broad host specificity causing disease in a variety of animals (Hook, 1990).

Non-typhoidal Salmonella infection is a common cause of food-borne illness worldwide. Several non-typhoidal serotypes of Salmonella cause salmonellosis in humans. Though some of these serotypes are the primary pathogens of lower animals their infecton in humans manifest as septicemia, meningitis or deep-seated pyrogenic infections (Ayyagiri et al., 1990). The number of cases each year is difficult to estimate, since in many instances the disease caused by these organisms is relatively mild and self-limiting. Yet, in susceptible individuals, such as very young, elderly and those immunocompromised, a common gastrointestinal disease can progress to a life-threatening septicemia. Also, the ubiquitous nature of these organisms makes them capable to infect a wide variety of animal species used
for food and contaminate various plant products, making human infections common. An early step in the pathogenesis of non-typhoidal *Salmonella* is the ability to penetrate the intestinal epithelium. Whether salmonellosis is confined to the intestinal form or progresses to systemic infection depends on the ability of the organism to invade and penetrate intestinal epithelial cells. Acquired through the food chain, *Salmonella* infect the ileum of colon (Finlay, 1994). The bacteria then translocate the epithelial barrier through M cells that overlie lymphoid follicles (Clark et al., 1994; Jones et al., 1994). They then subvert the normal function of M cells to invade the host cell. *Salmonella* then encounters the resident tissue macrophages that are intimately associated with the M cells and rather than being destroyed by these professional phagocytes after internalization, survives intracellularly (Fields et al., 1986). *In vitro* studies documented that infection of macrophages by several *Salmonella* species, including *S. Typhimurium* and *S. Typhi* leads to the death of phagocytes (Chen et al., 1996; Monack et al., 1996; Lindgren et al., 1996). Once inside the macrophages, *Salmonella* replicate and produce cytotoxins. The cell death that results from these cytotoxins has been linked to the Type III secretion system of serovar *S. Typhimurium* which is secreted to the phagosomal compartments (Chen et al., 1996) and this also prevents phagosome–lysosome fusion. The precise molecular mechanism for host-specific intramacrophage survival by various *Salmonella* serovars is still unclear (Schwan et al., 2000).

Though there are large number of reports that explain the infectivity of human adapted serovars like *S. Typhi*, very limited information is available on non-typhoidal serotypes like *S. Weltevreden*, *S. Bareilly*, *S. Virchow* and *S. Newport*. In this study, the interaction of the above said seafood serotypes of *Salmonella* with the murine macrophage cell line J774.A1 is being described. The ability of human-adapted serotype like *S. Typhi* (clinical) and *S. Paratyphi* (seafood) was also tested for their efficiency to invade and survive inside murine macrophages.

The emergence of antibiotic resistant strains of *Salmonella* is of great concern worldwide (Rowe et al., 1990), and is thought to be mainly due to the excessive use of antimicrobial agents in humans and animals. A small increase in the proportion of drug resistant strains of *Salmonella* was reported by the *Salmonella* Reference Center at the Institute of Medical Research in Kuala Lumpur from 1989 to 1994 (Yasin et al., 1995). Reports from other countries suggest that the proportion of multidrug resistant strains of *Salmonella* is on the rise (Guera et al., 2001; Szych et al., 2001; Chiu et al., 2002). Also, the isolation of ceftriaxone and ciprofloxacin resistant strains of *Salmonella* is of particular concern as these have become the drug of choice in the treatment of affected children (Chiu et al., 2002).
The important factor contributing to the development of resistance is the indiscriminate use of antimicrobials in human, veterinary medicine, animal husbandry, agricultural and aquacultural practices. Moreover, in animal husbandry antimicrobial agents are used both for treatment and growth promotion (Tollefson et al., 1997). Of recent concern is the prevalence of ACSSUT phenotype of S. Typhimurium DT104. Threlfall et al. (1993) reported that the prevalence of this phenotype has doubled between 1981 and 1989. Antimicrobial resistance was not found to be serotype specific (Zhao et al., 2001a). Many genetic elements have contributed to the development of resistance in bacteria. Besides chromosomal mutations, DNA mobile elements such as transposons and integrons have contributed to the rapid dissemination of resistance. These elements often spread by incorporation into plasmids and move from plasmid to chromosome where they are inherited by daughter cells. Integrons are mobile DNA, primarily found in Gram negative bacteria. They are the genetic elements which integrate by site specific recombination carrying gene cassettes, which usually confer antibiotic resistance. Three classes of integrons are characterized in detail and are involved in antibiotic resistance. Though the presence of class I integrons has been responsible for multi drug resistance in various Salmonella serotypes, a recent study of genetic characterization of antimicrobial resistance in S. Weltevreden did not find any presence of class I integron in any of their multi drug resistant strains (Aarestrup et al., 2003).

This study attempted the genetic characterization of nontyphoidal serotypes like S. Weltevreden, S. Paratyphi C and S. Anatum for the presence of different antibiotic resistant genes and class I integrons.

**OBJECTIVES OF THE PRESENT STUDY**

1. To study the efficiency of different primers to detect seafood associated Salmonella.

2. To study the presence of genes encoding putative virulence factors in seafood associated Salmonella.

3. To study the genomic diversity of different seafood isolates of Salmonella using molecular techniques.


5. To study the antibiotic resistance of Salmonella isolates by antibiogram and PCR to characterize the genetic basis of resistance.