introduction
1. INTRODUCTION

The modern history of cholera began in 1817. At that time, an epidemic outbreak was reported in India, which subsequently spread across the Indian continent and was then defined as the first pandemic outbreak of cholera disease in Southeast Asia. During the cholera epidemic in 1854 in Florence, the Italian physician Filippo Pacini (1812 to 1883) discovered the first *Vibrio* species *Vibrio cholerae*, the causative agent of cholera. Pacini’s important discovery was overshadowed by the work of Robert Koch (1843-1910), a German physician and bacteriologist who studied cholera during epidemics in India (Calcutta) and Egypt (Alexandria). In 1883, he conclusively isolated the causative agent of cholera from pond water during a cholera outbreak. Koch (1884) named the comma-shaped organisms Kommabazillen, and for several decades, the name *Vibrio comma* was used. When the important work of Pacini was recognized, the name was changed to *Vibrio cholerae* (Bik, 1996).

Accounts of a cholera-like disease go back to the times of Hippocrates and Buddha and perhaps even earlier (Barua, 1991). During the 19th century six cholera pandemics took place, ending in 1923 and affecting mostly the continents located in the southern hemisphere, as well as North America and Europe (Pollitzer, 1959 and Barua, 1991). In 1961, the seventh pandemic began in Indonesia, then spread to the Indian subcontinent and the Middle East, then moved on to Africa in the 1970s and finally reached South America in the early 1990s (Blake, 1994; Swerdlow and Isaacson, 1994; Tauxe *et al.*, 1994 and Faruque *et al.*, 1998).

*V. cholerae* is a motile, Gram-negative curved rod that belongs to the family *Vibrionaceae*. About 200 recognized “O” serogroups are known, however only
serogroup “O1” and the newly emerged “O139” have been associated with severe
disease and cholera pandemics. In contrast, intestinal and/ or extraintestinal infections
with non-O1 and O139 serogroups or non-toxigenic O1 strains are rarely found and
seem to be of little clinical significance (Morris, 1994 and Rodrigue et al., 1994).

The etiologic agents responsible for cholera which has been recognized as a
killer disease and many other infectious diseases such as tuberculosis, anthrax,
diphtheria, tetanus and typhoid fever were identified during the 19th century.
Although cholera had been prevalent in many parts of the world for centuries, it is
only during the last 50 years that we have learnt important aspects of the disease,
including its pathogenic mechanism, treatment, and prevention (Rabbani and
Greenough, 1999).

Interestingly, cholera is one of the few bacterial diseases known for its
pandemicity (Huq et al., 2005). During late 1992 in India and then in Bangladesh
appeared epidemic cholera like diarrhea. The early pandemics preceded Koch’s
discovery of the infectious cause of cholera, and thus have never been associated with
a specific strain variant. The causative bacterium failed to agglutinate with any of the
then existing 138 V. cholerae O antiserum (Albert et al., 1993) and was designated
O139 with the synonym “Bengal” to commemorate its emergence in the coast of Bay
of Bengal. Since then, O1 and O139 remains the two recognized serogroups causing
epidemics of cholera (Alam et al., 2006 and Shi et al., 2006).

The fifth and sixth pandemics, however, had been caused by the V. cholerae
serogroup O1 of biotype ‘Classical’, while the seventh pandemic was caused by
serogroup O1 biotype ‘El Tor’ (Barua, 1991). In 1992, a serogroup conversion event
led to the emergence of the new V. cholerae serogroup O139, resulting in a large
epidemic in Bangladesh and India (Albert et al., 1993; Ramamurthy et al., 1993 and Swerdlow and Ries, 1993). Preliminary epidemiologic reports documenting the severe nature of disease caused by O139, its rapid spread to neighboring countries and its ability to cause large explosive outbreaks (Public Health Laboratory Service, 1993 and Ramamurthy et al., 1993) give testimony that this new serogroups may represent the etiologic agent of a new, eighth pandemic of cholera. Since then O139 strains are endemically found in these areas and cases have been reported all over south-east Asia. This epidemic has already been described as the eighth pandemic (Faruque et al., 1998), however, *V. cholerae* O1 El Tor strains also still persist in these areas. All the eight pandemics occurred throughout the world is given in Table 1 (Kaper et al., 1995 and Bik, 1996).

Table 1. The eight cholera pandemics

<table>
<thead>
<tr>
<th>Pandemics</th>
<th>Period</th>
<th>Start</th>
<th>Regions affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1817-1823</td>
<td>India</td>
<td>Bengal, South East Asia, China, East Africa</td>
</tr>
<tr>
<td>2</td>
<td>1829-1851</td>
<td>Astrakhan</td>
<td>Middle East, Russia, Europe, North and Latin America, North Africa</td>
</tr>
<tr>
<td>3</td>
<td>1852-1859</td>
<td>India</td>
<td>Middle East, Europe, North and Latin America, North and East Africa, South East Asia, China</td>
</tr>
<tr>
<td>4</td>
<td>1863-1879</td>
<td>India</td>
<td>Middle East, Europe, North and South America, Africa, South East Asia</td>
</tr>
<tr>
<td>5</td>
<td>1881-1896</td>
<td>India</td>
<td>Europe, North and South America, Middle East, Russia, North Africa, South East Asia</td>
</tr>
<tr>
<td>6</td>
<td>1899-1923</td>
<td>India</td>
<td>Middle East, Russia, Europe, South East Asia, China</td>
</tr>
<tr>
<td>7</td>
<td>1961-1991</td>
<td>Sulawesi</td>
<td>South East Asia, India, Middle East, Africa, Turkey, Italy, Portugal, South and Central America</td>
</tr>
<tr>
<td>8</td>
<td>1992-1993</td>
<td>India</td>
<td>Pakistan, Nepal, China, Thailand, Kazakhstan, Afganistan, Malaysia</td>
</tr>
</tbody>
</table>
*V. cholerae* O-group 1 which causes cholera has historically been the *Vibrio* of great interest to clinicians, microbiologists, public health officials and epidemiologists. As a result of continuing scientific and medical effort directed toward combating cholera, major improvements in medical treatment as well as a better understanding of the molecular processes involved in the virulence of *V. cholerae* have been achieved.

However not until microbiologists began to sort out separate species from the "Wastebasket", NCV / NAG (Non-cholera Vibrios / Non-Agglutinable Vibrios) terminology became apparent that these Vibrios included several species that are often pathogenic for human and that have distinct clinical features, ecological niches, pathogenic mechanisms and epidemiologic characteristics. Despite all efforts, new variants of this pathogen are obviously still evolving, that are able to circumvent established immunity and to resist clinical and hygienic prophylactics.

Worldwide Vibrios are highly abundant in aquatic environments such as estuaries, marine and coastal waters, sediments, and aquaculture settings (Colwell and Spira, 1992; Rehnstam *et al*., 1993; Ortigosa *et al*., 1994; Colwell, 1996; Barbieri *et al*., 1999; Urakawa *et al*., 2000; Denner *et al*., 2002 and Heidelberg *et al*., 2002). Several independent studies have showed that Vibrios appear at high densities in marine organisms such as corals (Rosenberg and Ben-Haim, 2002), fish (Arias *et al*., 1995; Grisez *et al*., 1997; Ringo and Birkbeck, 1999 and Huys *et al*., 2001), molluscs (Sawabe *et al*., 2003), seagrass, sponges, shrimps (Vandenberghe *et al*., 1999; Gomez-Gil *et al*., 2000 and Vandenberghe *et al*., 2003) and zooplankton (Johnson and Shunk, 1936; Huq *et al*., 1983; Verdonck *et al*., 1994; Verdonck *et al*., 1997; Suantika *et al*., 2001 and Heidelberg *et al*., 2002a).
Interestingly, there is a correlation of cholera outbreaks and the seasonal occurrence of algal blooms (Epstein, 1993; Islam et al., 1994 and Colwell, 1996); however, there is no direct evidence that such events lead to an enrichment of toxigenic *V. cholerae* strains responsible for cholera epidemics. *V. cholerae* is closely associated with plankton and is assumed that cholera outbreaks are linked with planktonic blooms and the sea surface temperature and so such outbreaks may be predicted by monitoring these parameters by remote sensing (Lipp et al., 2002). Non-O1 and non-O139 strains are more frequently isolated from rivers and estuarine areas than O1 and O139 strains, and interestingly most environmental O1 strains are non-toxigenic (Colwell and Spira, 1992). This led to the hypothesis that environmental strains that acquire the respective virulence genes, which are harbored on genetic mobile elements, may then be enriched by intestinal conditions (Waldor and Mekalanos, 1996 and Karaolis et al., 1998). Recent evidence (Faruque et al., 1998) demonstrates that non-toxigenic environmental strains can be converted by phage transduction with cholera toxin (CT)-encoding phage CTXP, and this event could conceivably also take place in the gastrointestinal environment, yielding new detectable toxigenic strains.

Cholera a life-threatening illness, particularly among children, in countries with poor sanitation (Shi et al., 2006) caused by *V. cholerae* was the first pathogenic *Vibrio* species to be discovered and scientific opinion is divided on the epidemiology of *V. cholerae* infections. It is the aetiological agent of cholera, a severe diarrheal disease that occurs most frequently in epidemic form. Cholera has been epidemic in Southern Asia for at least 1000 years but also spread worldwide to cause seven pandemics since 1817 (Wachsmuth et al., 1994).
V. cholerae, exist as natural inhabitants of aquatic ecosystems (Colwell et al., 1977; Garay et al., 1985; Islam et al., 1994 and Colwell, 1996), thus making them facultative human pathogens. V. cholerae enters the human host via contaminated food and/or water (Wachsmuth et al., 1994). It moves along and attaches to surfaces with the aid of flagellum and pili, which may act as adhesions (Moorthy and Watnick, 2004). In the intestine, this bacterium adheres to the epithelium and produces an enterotoxin, cholera toxin (CT). This toxin causes an intense watery diarrhea that may lead to death, but it plays no role when V. cholerae is in the environment (Reidl and Klose, 2002). The organism also enters a “viable but non culturable” state under certain conditions (Roszak and Colwell, 1987).

The genus Vibrio includes both pathogenic and nonpathogenic strains that vary in their virulence gene content (Faruque et al., 1998). This bacterium contains a wide variety of strains and biotypes, receiving and transferring genes for toxins (Waldor and Mekalanos, 1996), colonization factors (Brown and Taylor, 1995 and Karaolis et al., 1999), antibiotic resistance (Hochhut and Waldor, 1999), capsular polysaccharides that provide resistance to chlorine (Yildiz and Schoolnik, 1999) and new surface antigens such as the O139 lipopolysaccharide and O antigen capsule (Waldor et al., 1994 and Bik et al., 1995). Furthermore, the fact is that pathogenic strains of V. cholerae are clonally distinct from environmental, non-pathogenic V. cholerae strains (Faruque et al., 2004).

Environmental studies show that these bacteria strongly influence nutrient cycling in the marine environment. Most medical investigators believe that V. cholerae of human origin spreads by faecal contamination of water and foods (Blake et al., 1980). Various species of this genus are also devastating pathogens for finfish,
shellfish and mammals. Recently, a number of reports have highlighted the pathogenic potential of Vibrios towards humans and marine animals (corals, gorgonians, and shrimps) which may be coupled with rising water temperature due to global warming (Sechi et al., 2000; Kushmaro et al., 2001; Martin et al., 2002 and Rosenberg and Ben-Haim, 2002). *V. cholerae* O1 is found associated with marine organisms and it has been shown that these strains can bind to chitin (Nalin, 1976), and in addition can then acquire acid tolerance (Nalin et al., 1979).

The potential severity of non-O1 *V. cholerae* infections is highlighted by Blake et al. (1980a) who described a case of fatal septicemia caused by *V. cholerae* which did not agglutinate with *V. cholerae* O1 antiserum. These strains also referred to as NAG or NCV, which have been recognized as causative agents of outbreaks and sporadic cases of gastroenteritis (Spira et al., 1981), although large epidemics O1 have not been reported. More than one type of gastroenteritis syndrome may be associated with *V. cholerae* non-O1; that is some strains of *V. cholerae* non-O1 cause an illness clinically indistinguishable from cholera, but others cause fever and bloody diarrhea (McIntyre et al., 1979 and Blake et al., 1980).

In the epidemiology of cholera, the role, if any of naturally occurring environmental *V. cholerae* is not clearly understood. Some workers believe that aquatic reservoirs of *V. cholerae* might be the mechanisms by which cholera endemicity is maintained in a given area and that the aquatic reservoir is probably the vehicle for primary transmission of infection (Colwell et al., 1981 and Miller et al., 1985). Though the importance of pathogenic bacteria especially *V. cholerae*, in tropical water has been realized, only a few investigations on their occurrence, distribution and pathogenicity in tropical marine environment have been carried out.
The clinical management of cholera has advanced over the past 40 years, however, cholera remains a serious threat in developing countries with poor sanitation, limited health care and unsafe drinking water. Hence, the present study has been carried out with the following objectives to understand the ecology and distribution of *V. cholerae* and the influence of abiotic factors on their abundance in Tuticorin coast, Southeastern India.

The present study was carried out to monitor the total heterotrophic bacteria, faecal coliforms and *V. cholerae* in the water column and sediments along with selected physico-chemical parameters, for a period of two years, thereby elucidating information on the distribution and seasonal variations of *V. cholerae* in Tuticorin coastal waters of Southeastern India. The seasonal incidence of *V. cholerae* in the harvested finfish and shellfish collected from the landing centers were explicated. The antibiotic resistance pattern of *V. cholerae* strains isolated from marine environment and seafood samples were elucidated. A study on the effect of herbal decoctions as preservatives for seafoods was also experimented. The impact of sediments on the landing centers was performed.

Much of the earliest interest in aquatic microbiology in India was oriented towards heterotrophic bacterial activities, biodegradation, fouling and microbial population related to public health. Microbiologist mostly focused their attention on the role of bacteria and their significance in seasonal replenishment of the nutrient present in the sea. Realizing the lacuna with respect to the ecology of autochthonous and allochthonous microorganisms in general and pathogens in particular, inhabiting a particular ecological niche, increased attention is being devoted to this aspect of late.