1. General introduction

1.1 Water: The elixir of life

Water that sustains life, is a natural resource, a basic human need and a precious natural asset. Despite its abundance this vast resource has come to exist as a scarce commodity. Access to safe water is a basic human need and a fundamental right as well as crucial for sustainable development, including the preservation of our natural environment and the alleviation of poverty and hunger. It is indispensable for human health and well-being. Quantity and quality of the Nation's water resources has been and will continue to be critically important to ensure, protection of life and property, economic well-being, and healthy ecosystems.

1.1.1 Environmental pollution and water quality

Environmental pollution, a potential global problem, has rendered waters along the coastline, recreational beaches, rivers, lakes, ponds, streams and ground water undesirable for public use. Contaminated water poses major health threat to human beings worldwide. The problem is particularly significant in developing countries which lacks proper water distribution infrastructure, and in arid areas where water sources are scarce, surface waters such as rivers, streams and lakes are used for multiple activities, including livestock watering, bathing, cooking and drinking.

Rapid urban and industrial growth has resulted in a vast quantity of potentially harmful waste products being released into the environment. The surface water bodies such as rivers, lakes, backwaters and estuaries are thus continuously subjected to a dynamic state of change. Additions of undesirable substances from various sources into the natural water body causes alteration in the physical, chemical and biological characteristics of water that lead to conditions which disturb the ecological balance. Thus, pollution results in deleterious effects, which are harmful to living resources, hazardous to human health, hindrance to aquatic activities, impairment of quality for use of water and reduction of amenities.
Growing population, inadequate infrastructure for proper treatment and disposal of sewage, lack of sanitary condition, poverty, poor planning, industrial pollution, over exploitation of natural water and natural disasters are the main reasons of pollution of water. Majority of Indian rivers are polluted due to drainage of sewerage, solid wastes and industrial effluents into the aquatic environment without any treatment. Millions of people living along the rivers are thus exposed to polluted water. Ground water is also becoming unfit for human consumption due to seepage of microorganisms from polluted surface waters.

1.1.2 Water and disease

Historically, water has played a significant role in the transmission of human diseases. Typhoid fever, cholera, diarrhea (diarrhoea), infectious hepatitis, bacillary and amoebic dysenteries and many varieties of gastrointestinal diseases could be transmitted by water. Contamination by sewage or human excrement causes the greatest danger to public health associated with drinking and recreational water. Direct discharge of domestic waste, leaching from poorly maintained septic tanks, and improper management of farm waste are suspected as the major sources of water-borne diseases (Huttly, 1990).

1.2 Faecal contamination

The contamination of water by pathogenic microorganisms has been a persistent public safety concern. Faecal pollution is usually regarded as the most prominent contributor of water-borne pathogens to waterways and constitutes a severe health risk to the human population all over the world, especially in developing countries where there is poor sanitation and hygiene. Surface water bodies are presumed to be more vulnerable to faecal contamination than groundwater reservoirs due to the absence of natural soil protection, filtration and possibly the short distance between the occurrence of contamination and water extraction. Urbanization generates increasing loads of faecal wastes discharged to natural water resources.
Usually in urban, peri-urban and adjacent rural areas, surface water bodies receive faecally polluted urban discharges that contain pathogenic microorganisms in such high numbers that exceeds the recommended limits for water to be used by man for purposes such as drinking, recreation, or irrigation of crops eaten raw. Wildlife, sewage effluents, failing septic systems and runoff from farm animal feedlots and agricultural lands are important sources of faecal contamination of water bodies. It is widely known that sewage contains many human enteric pathogens, which are frequently transmitted by ingestion or by contact with polluted water. The most important human enteric pathogens include bacteria such as *Salmonella*, *Shigella*, *Vibrio*, *Escherichia coli* O157: H7, protozoans such as *Giardia*, *Cryptosporidium*, and viruses such as *Hepatitis A* and *Norwalk* are discharged in large numbers into the aquatic environment through faecal discharges.

### 1.2.1 Sanitation, sewage and waste disposal

Around 1.1 billion people globally do not have access to improved water supply sources, whereas 2.4 billion people do not have access to any type of improved sanitation facility (Kindhauser, 2003). About 2 million children less than 5 years of age die every year due to diarrheal diseases. The most affected are the populations in the developing countries, living in extreme conditions of poverty, normally peri-urban dwellers or rural inhabitants. Among the main problems which are responsible for this situation are: lack of priority given to the sector, lack of financial resources, lack of sustainability of water supply and sanitation services, poor hygiene behaviours, and inadequate sanitation in public places including hospitals, health centres and schools. Providing access to sufficient quantities of safe water, the provision of facilities for a sanitary disposal of excreta, and introducing sound hygiene behaviours are of capital importance to reduce the burden of disease caused by these risk factors.

Human excreta are the principal vehicle for the transmission and spread of a wide range of diseases. Therefore, sanitation facility is an important barrier to get human beings exposed to pathogenic organisms. For example, vast majority of diarrheal diseases in the world (88%) is attributable to unsafe water, sanitation and hygiene (WHO/UNICEF,
2003). Approximately 3.1% of annual deaths (1.7 million) and 3.7% of the annual health burden (disability adjusted life years, DALYs) worldwide are attributable to unsafe water, sanitation and hygiene. Rural India is always deprived of proper sanitation measure. But, 28% of Indian population had sanitation coverage (15% of rural and 61% of urban population). Although water plays a direct or indirect part in their transmission, ‘unsafe’ sanitation practices and lack of environmental hygiene, rather than water itself, are at the root of the spread of these infections.

In developing countries, 90% of all sewerage is simply discharged into the environment without any treatment. In rural areas, sewerage systems and treatment facilities are practically non-existent. Poor treatment facility of sewage put whole population vulnerable to water-borne diseases. According to the Planning Commission, India, 80 percent of pollution in rivers is from human sewage. The amount of sewage generated by each of the 23 macro cities in India comes around 1500 million litres/day and only a fraction of this is properly treated while the rest flows into the rivers and other dumping grounds. Waste water from domestic sources (grey water) carry relatively less infective pathogens, but eventually get faecal contamination when it passes through open channel, which is frequently used for defecation by urban poor. The insufficient treatment facility of wastewater and sewage also cause pollution of river water bodies. Therefore, quality of wastewater treatment facility is of public health importance in cities and its surrounding areas.

1.2.2 Enteric pathogens

Enteric bacterial pathogens are facultative anaerobic gram-negative bacilli, whose natural habitats or reservoirs are the intestinal tracts of humans or animals. They include bacteria belonging to the families Enterobacteriaceae, Vibrionaceae and Pasteurellaceae of which the main pathogens of interest are Enterobacteriaceae and Vibrionaceae. Representative pathogens in Enterobacteriaceae are entropathogenic Escherichia coli, causing gastroenteritis or urinary tract infections; Salmonella, causing typhoid fever and gastroenteritis; Shigella, causing bacillary dysentery; Klebsiella, causing pneumonia and Yersinia, causing plague.
Representative pathogens in *Vibrionaceae* are *Vibrio cholerae*, causing cholera; *V. parahaemolyticus*, causing gastroenteritis in humans following consumption of contaminated seafood, and *V. anguillarum* and others, which are responsible for fish disease (Kaysner and DePaola, 2001). Almost all the mentioned enteric bacterial pathogens are implicated in water-borne diseases (Sharma *et al*., 2003). However, the quality of water has a great influence on public health, poor microbiological quality is likely to lead to outbreaks of infectious water related diseases and may cause serious epidemics.

1.2.3 Enteric infections: A global burden

Emerging water-borne pathogens constitute a major health hazard in both developed and developing nations. Water-borne diseases are common in all parts of the world where there is poor hygiene and sanitation. In developing countries, diarrhea is almost universally infectious in origin and spreads by means of contaminated food and water. Enteric infections (diarrhea) remain a major cause of morbidity and mortality worldwide particularly among children living in the developing world. Most commonly majority of infections are transmitted by the faecal-oral route through water, food and person-to-person transmission. The death toll from diarrheal diseases comes nearly 1-3 million per year, mostly infants and young children under 5 years of age, ranking third among all causes of disease burden worldwide (WHO, 2004). In addition, evidence has been emerging for long-term consequences of early childhood diarrheal disease on growth, physical and cognitive development that may translate into costly impairment of human potential and productivity (Guerrant *et al*., 2002). Furthermore, outbreaks of cholera, shigellosis and typhoid fever occur in resource-poor countries, affecting adults as well. Despite industrialization, wealth and public health interventions to ensure water quality and sewage disposal, intestinal infections have a major impact in the Western world, including both food-borne (*Salmonella* spp. and *Campylobacter* spp.) and water-borne infections (*enterohaemorrhagic E. coli*) (Wheeler *et al*., 1999). Enterotoxigenic *E. coli* and *Vibrio* spp. are the important cause of acute watery diarrhea in adults and children in developing countries. All the cholera cases and death reported to WHO in 2001, Africa accounted for 94%, followed by Asia, America and Europe (notified only imported cases).
However, globally the actual figures are estimated to be higher owing to under reporting and other limitations of surveillance systems and to the increased size of vulnerable populations.

1.2.4 Diarrhea and their burden in India

Acute diarrheal disease is one of the major public health problems, particularly in areas where cholera is still a disease burden. Although international efforts to control diarrheal diseases were launched by WHO (Synder and Merson, 1982), the Indian subcontinent is still known as the ‘home of cholera’ (Bhattacharya et al., 1996). Affecting children under the age of five years. The Planning Commission in its report - *India Assessment 2002 – Water Supply and Sanitation*, acknowledges that mortality and morbidity levels due to water-borne diseases in the country are unacceptably high, and diarrhea ranking top in morbidity and 10th in mortality. According to 10th five-year plan documents, 70-80% water related diseases are associated with water contamination and poor sanitation. About 80% children suffer from water related diseases every year and is more acute in rural India, where almost 70% of the people live. According to Central Bureau of Health Intelligence (CBHI, 2003a) among all reported communicable diseases in 2002, 29% shared by diarrheal diseases and in 2003 there were 10.5 million diarrheal cases with 4709 deaths (CBHI, 2003b) and 2893 cholera cases. India belongs to high endemic country of enteric fever and recent surveillance studies have shown that the incidence rate of enteric fever may peak as early as 1-5 years of age. In tropical countries, like India, mixed infection (infection by multiple organisms, particularly the combination of bacteria and microscopic parasites) is very common. According to world health report 1999, 8.2 per cent of DALYs lost in India is contributed by diarrheal diseases and 30.1 percent of global DALYs lost due to diarrhea is contributed by India alone.

1.2.5 Pollution indicator bacteria

The microbial examination is used worldwide to monitor and control the quality and safety of various types of waters like potable waters (water intended for drinking or use in food preparation), treated and untreated recreational waters (swimming pools, spa
pools, and hydrotherapy pools). Microbiological examination of water samples is usually undertaken to ensure the water is safe to drink or bathe in. As many potential pathogens could be associated with water and their concentration may be very low, it is thus impractical to screen samples for all possible pathogens. Instead, various indicator organisms have been used as surrogate markers of risk.

Since their first isolation from faeces in the late 19th century, the coliform group of bacteria has been used as an indicator of the bacteriological safety of water. The coliform group merits consideration as an indicator of pollution because these bacteria are always present in the intestinal tracts of humans and other warm-blooded animals and are excreted in large numbers in faecal wastes. Although the sanitary significance of some coliform strains is questionable, all members of the group may be of faecal origin, and it should be assumed that they are of faecal origin unless it can be proven otherwise. In fact, water is not a natural medium for coliform organisms, and their presence must at least be regarded as indicative of pollution in its widest sense.

The presence of coliform organisms in water has been attributed to influxes of allochthonous bacteria from waste discharges and surface water drainage. The proportion of faecal coliforms (FC) comprising the total coliform (TC) population or the total viable bacterial population can be used as an index of pollution from sanitary wastes of human or other animal origin. These organisms are considered as ideal indicator organisms as they meet the following criteria.

- It should be a member of the intestinal microflora of warm-blooded animals.
- It should be present when pathogens are present and absent in uncontaminated water.
- It should be present in greater number than the pathogens.
- It should be equally resistant as the pathogens to environmental insults.
- It should not multiply in the environment.
- It should be detectable by means of easy, rapid, and inexpensive methods.
- The indicator organisms should be non-pathogenic.
1.2.6 Total coliforms (TC)

The coliform or total coliform group includes all of the aerobic and facultative anaerobic, Gram negative, non-spore forming rod shaped bacteria that ferment lactose in 24-48 hours. This group includes the genera *Escherichia coli*, *Citrobacter*, *Enterobacter* and *Klebsiella*. From the health point of view, the coliform test has been the most important test used to analyse the quality of water. The total coliform test remains the primary indicator of bacteriological quality of potable water, distribution system water and public water supplies because a broader measure of pollution is desired for these waters.

1.2.7 Faecal coliforms (FC)

Faecal coliform is a sub group of total coliform and is defined as Gram negative, non-spore forming rods that ferment lactose in $24 \pm 2$ hours at $44.5 \pm 0.2^\circ C$ with the production of gas in multiple tube procedure. The major species in this group is *E. coli*, a species indicative of faecal pollution and the possible presence of enteric pathogens. The presence of these organisms particularly *E. coli*, indicates the possible presence of other disease causing bacteria. The rational behind this procedure is that *E. coli* is always present in sewage in higher concentration whereas pathogens are present in variable numbers. Thus there is a greater probability that *E. coli* will be present in water contaminated with raw sewage at any time than those pathogenic organisms detected. Thus it is assumed that if *E. coli* is present, other enteric pathogens are also present, if they are absent, disease-producing organisms are probably also absent.

1.3. *Escherichia coli*

As the pre-dominant species among the facultative anaerobic normal flora of the intestine, *E. coli* which belongs to the family *Enterobacteriaceae* has an important role in maintaining intestinal physiology (Siitonen, 1992). This organism was first described by German paediatrician Theobald Escherich in 1885, under the name *Bacterium coli commune* as a short, plump rod that had initially been isolated from normal infant faeces (Escherich, 1885). However, he regarded them to be harmless saprophytes. For more than half a century, *E. coli* was considered a major commensal in faeces which was avirulent. This view...
has changed progressively over the years with accumulation of evidence indicating that \textit{E. coli} is capable of causing disease in man (Nataro and Kaper, 1998).

\subsection*{1.3.1 \textit{Escherichia coli}: An emerging pathogen}

\textit{E. coli} was first suspected as being a cause of children’s diarrhea in the 1940s, when nursery epidemics of severe diarrhea were found to be associated with particular serotypes of \textit{E. coli} (Bray, 1945). Now, diarrhea is a major cause of morbidity worldwide, and remains the leading cause of childhood mortality in the developing world. It was estimated that 88\% of that burden is attributable to unsafe water supply, sanitation and hygiene. Enteric bacteria comprise the major etiologic agents of sporadic and epidemic diarrhea both in children and adults. Detection of etiological agents of diarrhea is important for therapeutic aspects and for implementing appropriate control strategies. In developing countries, the bacterial pathogen most commonly associated with endemic form of diarrhea is diarrhegenic \textit{E. coli} (DEC).

\subsection*{1.3.2 Diarrhegenic \textit{E. coli}}

Virulent strains of \textit{E. coli} which cause diarrhea are referred to as diarrhegenic \textit{E. coli}. Most \textit{E. coli} strains are harmless commensals but others are pathogenic. Differentiation of the pathogenic strains from the commensal ones was accomplished on the basis of virulence properties, mechanisms of pathogenicity, clinical syndromes and serotyping of distinct “O” (somatic), “H” (flagellar) and “K” (capsule) antigens (Doyle \textit{et al.}, 1997; Wilshaw \textit{et al.}, 2000). Due to the ease of access of pathogens ingested with food, the human gastro-intestinal tract is susceptible to diarrhegenic \textit{E. coli} infections. Several \textit{E. coli} pathogens have been implicated with diarrheal illness which includes enteropathogenic \textit{E. coli} (EPEC), enterotoxigenic \textit{E. coli} (ETEC), enteroinvasive \textit{E. coli} (EIEC), enterohemorrhagic \textit{E. coli} (EHEC) and enteroaggregative \textit{E. coli} (EAggEC) constitute a very large and important group of water-borne pathogens. Each of the pathotypes has distinguishing characteristics related to epidemiology, pathogenesis, clinical manifestations and treatment.
1.3.2.1 Enterotoxigenic *E. coli* (ETEC)

The history of enterotoxigenic *E. coli* begins in 1956 in Calcutta from children and adults with a cholera-like illness (De et al., 1956). ETEC were first identified as a cause of diarrhea in 1970 and are now considered as a major cause of *E. coli* associated diarrhea worldwide. The symptoms of ETEC infection are due to the production of one or both of two types of enterotoxins by the bacterium, namely heat stable enterotoxins (ST) and heat labile enterotoxins (LT). ST’s are small, monomeric plasmid mediated toxins comprising two unrelated classes, namely STa (or ST-I) and STb (Sooka et al., 2004). The receptor for STa is guanylate cyclase C and binding results in increased intracellular cyclic guanyl monophosphate (cGMP) levels leading to stimulation of chloride secretion or inhibition of sodium chloride absorption and subsequent intestinal fluid secretion (Crane et al., 1992). ETEC are associated with poor hygiene and sanitation and are therefore of high importance in developing countries. In such countries the number of diarrheal episodes due to ETEC is equivalent to that caused by EPEC. Many individuals visiting developing countries suffer from ETEC infection during their stay or upon their return. Most of these cases probably occur due to the ingestion of water or food contaminated with ETEC by faecal matter and outbreaks can occur in hotels after person-to-person spread (Nataro and Kaper, 1998). The illness is known as traveler’s diarrhea. The percentage of cases of sporadic endemic infant diarrhea which are due to ETEC usually varies from 10 to 30%.

1.3.2.2 Enteropathogenic *E. coli* (EPEC)

In particular, EPEC was the first strain of *E. coli* incriminated as the cause of outbreaks of infantile diarrhea in the 1940s and 1950s (Bray, 1945). EPEC remain a major cause of infantile diarrhea worldwide, mostly in the developing world. A number of serotypes are responsible for disease and they possess a number of virulence factors although they do not produce any classic toxins. It is now known that EPEC possesses a virulence plasmid and numerous chromosomally encoded genes which together are responsible for virulence factors such as bundle-forming pili, secreted proteins and intimin (Chen and Frankel, 2005). It has been shown that these mechanisms are responsible for the disease caused by EPEC. However, the organization and regulation of the genes
involved is complex and has not yet been fully elucidated. The main virulence mechanism of EPEC appears to be the localized adherence of the bacterium to the host gut epithelium followed by the production of a characteristic ultra structural lesion whereby bacteria attach intimately and cause localized destruction of the intestinal brush border microvilli and distortion of the apical enterocyte membrane (Sooka et al., 2004). This results in cytoskeletal rearrangement and the formation of an actin rich cup like indentation (pedestal) at the site of bacterial contact. This was subsequently termed the attaching and effacing (AE) lesion (Sooka et al., 2004). The production of the AE lesion is followed by the injection of various components into the host cell via a type III secretion apparatus which leads to interruption of host cell activity (Sousa, 2006). As with other diarrhegenic E. coli strains, transmission of EPEC is faecal-oral, with contaminated hands, contaminated weaning foods or formula, or contaminated fomites serving as vehicles. In the uncommon adult outbreaks, water-borne and food-borne transmission has been reported. The reservoir of EPEC infection is thought to be symptomatic or asymptomatic children and asymptomatic adult carriers, including mothers and persons who handle infants.

1.3.2.3 Enterohemorrhagic E. coli (EHEC)

EHEC belong to a number of ‘O’ serogroups, but those serogroup O157 are the most important in human disease. The organism produces a toxin which has cytotoxic activity on Vero cells (African green monkey kidney cells) and is often termed Vero cytotoxin (VT) but may be also be known as Shiga-like toxin. EHEC are therefore also termed verocytotoxigenic E. coli (VTEC) and Shiga toxin-producing E. coli (STEC). EHEC also possesses a number of other mechanisms, such as adhesions, which are involved in virulence. EHEC was first identified as a human pathogen in 1982 after two outbreaks occurred in the USA (Kudva et al., 1998). Since then outbreaks have been reported from various parts of the world including North America, Western Europe, Australia and Asia. Ruminants, especially cattle, are the main reservoir of STEC, which are transmitted to humans primarily via contaminated foods and water (Karmali, 1989; Nataro and Kaper, 1998; Griffin and Tauxe, 1991; Griffin, 1995; Karch et al., 1999). Human infection with
EHEC is associated with a range of symptoms from non-bloody diarrhea, fever and vomiting through to hemorrhagic colitis and hemolytic uremic syndrome (HUS). HUS is characterized by acute renal failure, hemolytic anaemia and thrombocytopenia (Sousa, 2006). Between 2% and 7% of infected individuals usually develop HUS and it is more common in young children. The infectious dose has been estimated to be less than 100 organisms as determined from outbreak investigation (Sousa, 2006).

1.3.2.3.1 *Escherichia coli O157:H7* (EHEC)

During the past decade, *E. coli* O157:H7 has evolved from a clinical novelty to a global public-health concern. The organism was first recognised as a human pathogen in 1982, when it was implicated in two outbreaks of haemorrhagic colitis (Riley *et al*., 1983), a distinctive clinical entity characterised by abdominal cramps, bloody stools, and little or no fever. Since its identification, *E. coli* O157:H7 has been isolated in numerous worldwide outbreaks of hemorrhagic colitis and hemolytic uremic syndrome. The public health impact of EHEC infections is high because of their systemic complications, such as HUS, an important cause of acute renal failure in childhood, and late sequelae (Siegler, 1995; Andreoli *et al*., 2002), and their ability to cause large outbreaks. Contaminated drinking water and recreational water have been associated with outbreaks of haemorrhagic colitis caused by *E. coli* O157:H7. Outbreaks have been reported in many developed countries, including Canada, Europe, Australia, and Japan and the Centers for Disease Control and Prevention (CDC, 2000) has estimated that *E. coli* O157:H7 causes nearly 75,000 human infections in USA each year (Mead *et al*., 1999). Serotype O157:H7 has been implicated in most outbreaks and in most cases led to HUS globally (Karmali, 1989; Nataro and Kaper, 1998; Karch *et al*., 1999). There is growing concern about the risk to human health associated with non-O157 STEC serotypes (Tarr and Neill, 1996), over 200 of which have now been associated with human illness (World Health Organisation, 1999).

1.3.2.4 *Enteroaggregative E. coli* (EAggEC)

This bacterium was described in 1987, and identified in a child from Chile with persistent diarrhea (Nataro, 2005). EAggEC is increasingly recognized as an emerging
enteric pathogen. EAggEC are a more recent addition to the diarrhegenic classes of \textit{E. coli} and are so named due to their adherence pattern to cultured epithelial cells. Such an adherence pattern provides the gold standard for the definition of EAggEC and is seen as bacteria adhering in a “stacked brick-like” formation on the cell (Sousa, 2006). EAggEC are a significant cause of diarrhea in developing countries and are epidemiologically associated with acute or persistent diarrhea, the latter of which is of particular significance in children (Nataro and Kaper, 1998). Furthermore, EAggEC are a cause of diarrhea in travellers who have visited countries endemic for EAggEC. EAggEC do not induce attaching/effacing lesions characteristic of enteropathogenic \textit{E. coli} or produce cholera-like or shiga-like toxins characteristic of \textit{Vibrio cholerae} or \textit{Shigella dysenteriae} respectively. Little is known of their pathogenicity except that they produce a toxin known as the enteroaggregative heat-stable enterotoxin (EAST1) and that their aggregative adherence phenotype is mediated by at least two fimbriae (Czeczulin \textit{et al.}, 1997). The EAST1 enterotoxin is partly homologous and functionally similar to, but distinct from ST, and is thought to be responsible for the symptoms of infection. The association of EAggEC with diarrheal disease appears to be geographic. On the Indian subcontinent, EAggEC is important in paediatric diarrhea.

1.3.2.5 \textbf{Enteroinvasive \textit{E. coli} (EIEC)}

Enteroinvasive \textit{E. coli} is an important cause of diarrheal disease. They are responsible for a bacillary dysentery-like illness and, not surprisingly therefore, share some of the virulence properties associated with \textit{Shigella dysenteriae} (Sooka \textit{et al.}, 2004). The bacteria multiply within these cells eventually causing their death and the bacteria are released back into the colon after which they invade other cells. This process results in an inflammatory response accompanied by necrosis and ulceration of the large bowel leading to release of blood and mucus in the stool. The ability of EIEC to invade, survive and multiply within colonic enterocytes is dependent upon the presence of a 120-140 MDa plasmid which encodes all the genes necessary for such virulence including outer membrane proteins required for invasion (Sooka \textit{et al.}, 2004). Infection with EIEC results in a diarrheal illness characterized by a bloody, mucoid diarrhea.
1.4. **Salmonella**

*Salmonella* is a Gram-negative facultative rod-shaped bacterium which belongs to the family *Enterobacteriaceae*. *Salmonella* spp. are ubiquitous enteric bacteria and is a large genus including more than 2,400 serotypes (Nair et al., 2002; Martinez-Urtaza et al., 2004). All these serotypes are pathogenic to humans and cause a range of symptoms from mild gastroenteritis to severe illness or death. *Salmonella* are capable of infecting a large variety of both cold and warm-blooded animals. It is the etiologic agents of food-borne salmonellosis and also the agents that cause typhoid and paratyphoid fevers, resulting in an annual incidence of about 17 million cases worldwide (Kindhauser, 2003). The major *Salmonella* serotypes which are strictly adapted to humans or higher primates are *Salmonella enterica* serotypes *Typhi*, *Paratyphi A*, *Paratyphi B*, and *Paratyphi C*. India has the highest incidence, around three million cases each year (Anon, 2003, Typhoid, in *The Hindu*, February 23). Both typhoid pathogens are passed in the faeces and urine and people become infected after eating food or drinking beverages that have been handled by a person who is infected or by drinking water that has been contaminated by sewage containing the bacteria.

Once the bacteria enter the person’s body they multiply and spread from the intestine, into the bloodstream. Even after recovery from typhoid or paratyphoid, a small number of individuals (called carriers) continue to carry the bacteria (Ashbolt et al., 2004). *Salmonella* serotype *Enteritidis* is currently the main cause of human salmonellosis in most industrial countries (Stanley et al., 1992; Baggesen et al., 1997) where human infections are associated with consumption of contaminated food. Raw food of animal origin and cross-contamination of ready-to-eat products are the main vehicles for infection. *Salmonella* spp. are constantly found in environmental samples, because they are excreted by humans, pets, farm animals and wild life. Municipal sewage, agriculture pollution, and storm water runoff are the main sources of these pathogens in natural waters. It has been suggested that the survival capacity of environmental strains may depend on species and pollution sources (Polo et al., 1998; Baudart et al., 2000).
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1.5 Vibrio parahaemolyticus

*Vibrio parahaemolyticus* is a Gram-negative, halophilic asporogenous rod that is straight or has a single, rigid curve and distributed in temperate and tropical coastal waters throughout the world. *V. parahaemolyticus* was first identified as a human enteric pathogen in 1951 when Fujino *et al.* (1953) reported on a "shirasu" food poisoning outbreak in Japan. Since that time many investigators have examined the distribution of this species in natural environments, and showed that, unlike most enteric bacterial pathogens, they are found to occur in marine environment and contaminate fishery products especially during warm periods (Duan and Su, 2005). Epidemiological studies have established *V. parahaemolyticus* as a worldwide agent of gastroenteritis, and results of ecological studies demonstrated that it could be isolated from seafood, as well as estuarine, neritic, and brackish waters (Thompson *et al.*, 2004).

Consumption of raw or undercooked seafood, particularly shellfish, contaminated with *V. parahaemolyticus* may lead to the development of acute gastroenteritis characterized by diarrhea, headache, vomiting, nausea, abdominal cramps and low fever. This bacterium is recognized as the leading cause of human gastroenteritis associated with seafood consumption in the United States and an important seafood-borne pathogen throughout the world (Kaysner and DePaola, 2001). Most outbreaks of *V. parahaemolyticus* infections were caused by *V. parahaemolyticus* of diverse serotypes. However, increased incidences of gastroenteritis caused by *V. parahaemolyticus* serotype O3:K6 have been reported in many countries since 1996 (Chiou *et al.*, 2000). This serovar was first identified during a hospital-based active surveillance study of *V. parahaemolyticus* infections in Calcutta, India between 1994 and 1996 (Okuda *et al.*, 1997). The survey identified a sudden increase in incidences associated with this serovar, which accounted for 63% of total *V. parahaemolyticus* strains isolated from patients in Calcutta between September 1996 and April 1977. This highly virulent strain was subsequently recovered at a high rate in other Southeast Asian countries and was isolated from travelers arriving in Japan from various countries in the Southeast Asia (Okuda *et al.*, 1997; Chiou *et al.*, 2000; Vuddhakul *et al.*, 2000).
1.6 Vibrio cholerae

The agent responsible for cholera was first described in 1854 in Italy by Pacini, who found a large number of curved bacteria in the intestinal contents of cholera victims which he called Vibrio cholera. This initial discovery was overshadowed by the work of Robert Koch, who studied cholera in Egypt and demonstrated in 1883 that cholera was caused by this comma-shaped organism. Koch named the organism Kommabazillen and the subsequent name Vibrio comma was used for several decades before the pioneering work of Pacini who changed the species name as Vibrio cholerae (Thompson et al., 2004).

Vibrio spp. are classified in the family Vibrionaceae along with Aeromonas, Photobacterium, and Plesiomonas spp. V. cholerae are natural inhabitants of a wide range of aquatic ecosystems, including estuarine and coastal waters. Cholera is a highly epidemic diarrheal disease which continues to devastate many developing countries where socioeconomic conditions are poor, sanitary systems and public hygiene are rudimentary, and safe drinking water is not available (Girard et al., 2006). Cholera is usually transmitted to humans by ingestion of contaminated water and foods. The major virulence factor produced by V. cholerae is the cholera enterotoxin (CT). Of the several recognized serogroups, V. cholerae O1 has been responsible for these pandemics. In 1992 however, a new strain called V. cholerae O139 Bengal appeared in South India and caused explosive outbreaks of cholera-like disease (Ramarmurthy et al., 1993). In a matter of one year, the new strain spread to several parts of India and to neighbouring Bangladesh and Thailand (Nair et al., 1994). By the end of 1993, cholera outbreaks due to V. cholerae O139 were reported from South Asia and other countries of the world. Soon after its appearance, V. cholerae O139 outnumbered V. cholerae O1 and became the dominant serogroup in India and other parts of the Indian subcontinent (Girard et al., 2006). It was thought that this was probably the beginning of a new pandemic, the eighth pandemic of cholera (Nair et al., 1996). Except for the seventh pandemic, which originated in the island of Sulawesi in Indonesia, the other six pandemics arose from the Indian subcontinent, usually from the Ganges delta in Bengal. But, by 1994, there was a dramatic decrease in V. cholerae O139
and once again *V. cholerae* O1 became the dominant species. It is thought that, in the years to come, *V. cholerae* O139 is going to play an important role in the global epidemiology of cholera (Garg et al., 1998).

### 1.7 Survival of enteric bacteria in aquatic environments

The fate and long term survival of enteric bacteria in natural aquatic ecosystems has been of interest to public health. An understanding of the survival of faecal indicator organisms and the enteric pathogens in water is basic to the meaningful interpretation of sanitary water quality data. Although, detection of indicator bacteria suggests occurrence of pathogenic organisms in water, the potential health hazard is dependent on retention of critical density levels and associated virulence for the pathogens in a given time frame during transmission via the water route. Surface waters, such as rivers, lakes and seas, receive large quantities of wastewater from industrial, agricultural and domestic sources, including municipal sewage treatment plants. These surface waters, which contain many unknown compounds, are used as a source of drinking water, as well as for agricultural, recreational and religious activities around the world. Consequently, water pollution could be a serious public health and aquatic ecosystem problem.

Most sanitary indicator organisms as well as the enteric water-borne pathogens are bacteria whose natural environment is the intestine of man and warm-blooded animals. When discharged in the faeces, these microorganisms frequently gain entry into the water bodies. Once these bacteria deposited into the water, they are in an environment that is not favourable to their maintenance and viability. Therefore, understanding the survival of faecal indicator organisms and the enteric pathogens in water is basic to the meaningful interpretation of sanitary water quality data which is of interest to public health.

When allochthonous enteric bacterial species enter into the aquatic environments, they are not able to grow in natural waters, and they die off after a short time period, due to the influence of several biotic and abiotic factors which vary depending on the kind of water and the prevailing environmental conditions. The most important biotic and abiotic environmental factors influence the survival of these organisms are sunlight, temperature,
pH, salinity, nutrients, hydrostatic pressure, sedimentation, protozoan grazing, bacteriophages, competition, antibiotics and toxins.

1.7.1 Exposure to Sunlight

Light is considered to be the single most important contributor to bacterial die-off in the aquatic environments. Ultra violet (UV) light that reaches the earth is classified into two components, UV-A (400 to 320 nm) and UV-B (320 to 280 nm). The UV-B (280-320 nm) portion of the solar spectrum is the most bactericidal, causing direct photobiological DNA damage. At higher wavelengths, photochemical mechanisms become more important, usually acting through photosensitizers and tending to be more injurious in the presence of oxygen. It has been reported that the bactericidal action of sunlight on water is dependent on dissolved oxygen levels (Curtis et al., 1992; Reed, 1997). Photons of light are absorbed by photosensitizers, which become electronically excited and react with neighbouring oxygen molecules leading to the production of highly reactive oxygen species, singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radicals. Such species react with DNA causing both strand breakage and base changes (Acra et al., 1990). Strand breakage is usually lethal while base changes may result in a block in replication and other mutagenic events. Faecal microorganisms differ in their sensitivity to light in water and greater sunlight exposure was required to inactivate Enterococci compared to faecal coliforms, the latter group was also found to be more sensitive to light inactivation than faecal bacteriophages (somatic coliphages, F3 DNA phages, F3 RNA phages and Bacillus fragilis phages). Turbidity of the water column plays an important role in the transmission of light. Light was also demonstrated to exert a significant negative effect on the culturability of microorganisms which makes the detection impossible by means of traditional counting methods on plates, although these nonculturabale cells can maintain their infective capacity and pathogenic potential (Singh and McFeters, 1987).

1.7.2 Protozoan grazing and competition

Planktonic bacteria play an important role in nutrient recycling, organic matter decomposition and biomass formation in aquatic systems, thus forming a relevant link in
pelagic microbial food webs. Among the major biological mechanisms that have been implicated in affecting enteric bacterial concentrations in water are predation, and competition. Free living bacteria, their major predators, and bacterivorous protists, have coexisted in several types of habitats over long geological periods. Environmental bacteria are an integral part of microbial food webs and, as such, are constantly confronted with a range of predators and parasites such as protozoa and phages. Consumption by protozoa is considered to be a major source of bacterial mortality in most, freshwater and marine ecosystems. Due to their mobility, small size and generally high abundance, protozoa can track down ‘hot spots’ of bacterial growth even in the sparse vastness of the oligotrophic ocean or the narrowest soil crevices. Moreover, predation by bacterivorous protists in aquatic habitats can influence the morphological structure, taxonomic composition and physiological status of bacterial communities. As a result, genotypic and phenotypic composition can vary remarkably over time and space in bacterial communities. Grazing by phagotrophic protists, especially bacterivorous nanoflagellates and ciliates has been identified as a significant factor modifying bacterial populations in aquatic ecosystems and thus implicated as major trophic links between the microbial loop and the classical food chain (Hahn and Hofle, 2001).

1.7.3 Bacteriophages

Over the past 15 years, it has been realised that viruses are an important component of aquatic microbial food webs. They have been shown to be important controlling agents in planktonic community composition, diversity and succession, playing a key role in cell mortality and nutrient cycles. Bacterial viruses, or bacteriophages have numerous roles in marine systems.

Many studies detected coliphages in marine waters subject to sewage contamination. It was also shown that phages detected in seawater were active against *E. coli, Aerobacter aerogenes*, and *Serratia marinorubra*. The presence of coliphages was investigated in relation to their usage as indicators for faecal contamination, and in many cases a positive correlation between faecal phages, enteric viruses, and other pathogens has been
recorded. Nevertheless, the presence of enteric bacterial infectious phages does not necessarily indicate their actual activity in removing coliforms from marine water. Viruses lyse pelagic bacteria and it has been estimated that viruses can be responsible for >50% of the bacterial mortality in many ecosystems, thus affecting the flow of energy, carbon and bacterial community composition within the ecosystem (Fuhrman and Noble, 1995; Hennes and Simon, 1995; Mathias et al., 1995; Weinbauer and Höfle, 1998; Wilhelm and Smith, 2000; Fischer and Velimirov, 2002; Jacquet et al., 2005). In marine ecosystems, for example, Suttle and Chen (1992) estimated that 4-13% of the bacterial community would have to be infected daily and that 8-26% of the bacterial mortality could be explained by viral lysis in marine ecosystems. In freshwater ecosystems, viruses have also been shown to play a crucial role in bacterial mortality, with daily bacterial removal reaching on occasion up to 97% (Weinbauer and Hofle, 1998).

1.7.4 Antibiotics and toxins

Wide ranges of microbes are capable of exhibiting bacteriolytic and antibiotic activity (Nair and Simidu, 1987). Antibiosis occurs when the pathogen is inhibited or lethally affected by metabolic product of an antagonist such as enzyme, acid agents, or antibiotics (Maier et al., 2000; Sharma, 2000). Many marine free-living and sediment inhabiting marine bacteria have been shown to produce secondary metabolites that display antibacterial property (Burgess et al., 1991). Bacterium - bacterium antagonistic interactions involving antibiotic are well documented in soil and has been detected only in association with organic rich environment (e.g. seed, rhizosphere and straw fragment in soil) (Wright, 1956; Thomashow et al., 1990).

1.8 Survival of enteric bacteria in aquatic sediments

Aquatic sediments are a well-recognized reservoir for enteric microorganisms. Once pathogens enter into the water column, they may become associated with various suspended solids that eventually settle out and accumulate in the underlying sediments. Both bacteria and viruses possess electrostatic charges, which facilitates their adsorption onto fine-grained high-organic charged clays and muds. Several studies, focusing on the
occurrence of coliforms, faecal coliforms or *Enterococci* in estuarine water and coastal sediments, revealed that greater numbers (100 - or a 1000 - fold higher) of these organisms are found in the sediments than in the water above them. This phenomenon had been the impetus for detailed studies determining that these organisms indeed survive better in sediments than in the water. Soil and sediment particles are believed to function as microecological niches in which bacterial species can survive and perhaps replicate. Microbial sediment densities are dependent on numerous factors; nearby contamination sources and grain size are most significant. Clay and silts of same grain sizes usually have more organic matter and increased surface area, thus the occurrence of higher microbial numbers with longer survival than in sandy sediments. Enteric microorganisms are mainly accumulated in the surface layers of sediments where the concentration is 100-1000 times and higher than in overlying water. High concentrations occurred in the surface layer in comparison to the underlying sediment.

Due to the accumulation of pathogens in bottom sediments, resuspension of sediments can result in desorption of pathogens from sediments and the subsequent contamination of the overlying surface waters. Activities that resuspend sediments such as storm water events, wave action, tides, recreational use, and dredging can lead to transient increases in pathogens and pathogen indicators in the water column. In coastal waters this may indicate a potential increase in the risk of infection to human beings because of the resuspension of potentially pathogenic microorganisms from the surface sediment layer during recreational activities (Ferguson *et al.*, 2005).

1.9 Antibiotic resistance

Antibiotics have been rightly called miracle drugs but sixty years of use and misuse of antibiotics has resulted in increased frequencies of resistance for most combinations of antibiotics and bacteria. In fact, this bacterial adaptive evolution has been so successful that certain bacterial infections are almost untreatable with antibiotics. For any resistance mechanism, the bacterium is not only altered in its ability to withstand the drug but potentially also in its interaction with the host and environment. According to WHO the
resistance to antibiotics is an ability of bacterial population to survive the effect of inhibitory concentration of antimicrobial agents and multiple antibiotic resistance is the ability of bacterial population to survive the effect of inhibitory concentration of more than two antimicrobial agents.

The emergence and dissemination of antimicrobial resistance in bacteria to many classes of drugs is a serious public health issue worldwide. The main risk factor for this increase in resistance is the extensive and increased use of antibiotics in clinical medicine for preventing disease and promoting growth in animal husbandry. As a consequence, antimicrobial-resistant bacteria are selected for favouring antimicrobial resistant phenotypes thereby posing a critical public health threat in antimicrobial treatment efficiency. This has inevitably led to the emergence and dissemination of resistant bacteria and resistance genes in the environment. Being a potential public health hazard to the persons exposed to these aquatic environments through various activities, the prevalence of such antimicrobial-resistant strains in the environment is gaining worldwide concern. During the past few years, the distribution of many antimicrobial-resistant bacterial strains in aquatic environments has been observed in different regions of the globe (Niemi et al., 1983).

Normal intestinal flora of humans and animals constitute a major reservoir of antibiotic resistance genes for potentially pathogenic bacteria and may serve as chief indicators of selection pressure exerted by anti-microbial use in a given population. As a result of exposure to antibiotics, the level of resistance against antibiotics among bacteria belonging to the normal intestinal flora of humans and animals increases. The principle behind the development of resistance is that bacteria in the guts of humans and animals are exposed to different types, concentrations, and frequencies of antimicrobial agents. Over time, selective pressure selects resistant bacteria that have specific fingerprints for resistance to the antimicrobial agents that have been used. Thus, use of a particular antibiotic in human hosts, or in an environment may increase the risk of colonization by or, infection with resistant organisms in other humans or even animals that have not received that set of antibiotics but are sharing common environment with the humans. It
also increase the density of resistant organisms within a host that already harbour such organisms at a lower density resulting in enhanced shedding of these organisms and increased risk to other hosts. Resistant bacteria from the intestinal flora of animal and human reach the environment through faecal matters and again to reach the intestinal tract of humans directly through the ingestion of water contaminated with antimicrobial resistant bacteria, or indirectly through the exposure to environment or food.

1.9.1 Multiple antibiotic resistance (MAR)

In 1959, Watanabe discovered that some *E. coli* strains could transfer antibiotic resistance to antibiotic-sensitive strains of *Shigella* spp. and subsequent research has demonstrated that bacteria carrying transmissible R-factors are responsible for the spread of multiple antibiotic resistance among members of the *Enterobacteriaceae* (such as *E. coli*, *Salmonella typhi*, and *Shigella dysenteriae*), *Aeromonas* and *Yersinia* species, *Pseudomonas aeruginosa*, and *Vibrio cholerae*. Transmission of R-factors in the *Enterobacteriaceae* usually occurs by conjugation, which involves a specialized structure called the ‘sex pilus’ and requires cell-to-cell contact or cell-pilus-cell contact (Niemi *et al.*, 1983). The ability and the efficiency of different bacterial strains to donate or receive R-factors varies. Transmission of R-factors by conjugation is rapid and may spread rapidly among bacteria.

1.10 General objectives

1. To monitor the prevalence of faecal indicator bacteria such as *E. coli* and enteric pathogens such as *Salmonella*, *V. parahaemolyticus* and *V. cholerae* in water from different stations on either sides of the salt water regulator (Thanneermukkom barrage) in Kumarakom region of Vembanad estuary.

2. To monitor the prevalence of faecal coliform and *E. coli* in sediment from different stations on either sides of the salt water regulator in Kumarakom region of Vembanad estuary.

3. To find out the risk associated with *E. coli*, *Salmonella*, *V. parahaemolyticus* and *V. cholerae* isolated from the lake by antibiotic resistance analysis.

Diversity and survival of diarrhegenic *E. coli* and enteric pathogens in Vembanad lake with special reference to Kumarakom area
4. To study the survival of *E. coli*, *S. paratyphi*, *V. parahaemolyticus* in water and sediment as a function of biological factors (predation, competition and antibiosis).

5. To study the survival of *E. coli*, *S. paratyphi*, *V. parahaemolyticus* in water as a function of sunlight and ultraviolet (UV) light.

6. To study the effect of sunlight on the survival of plasmid bearing and plasmidless *E. coli* in estuarine water.

7. To study the *in situ* survival of *E. coli*, *S. paratyphi*, *V. parahaemolyticus* in estuarine water using membrane diffusion chamber.