CHAPTER 1

General introduction
Chapter 1

GENERAL INTRODUCTION

Robert Koch in 1979 postulated that microorganisms could be responsible for the outcome of diseases in the human host, and than after some years he has proved the association between microbes and diseases like cholera and tuberculosis and got the Nobel Prize for the latter in 1905 (Porter 2001). After some years Dr. Bizzozero who was an anatomist by profession in Turin, observed Gram-negative spirochetes within partial cells and gastric glands from dog tissue. We now know that he has discovered the Helicobacter species (Marshall, 2001).

Several observations by pathologists of spirochetes in human stomach tissue have been reported since. However until the early 1980s correlation between the bacterium *Helicobacter pylori* and gastro duodenal diseases was not established (Doenges, 1938; Freedburg and Barron, 1940; Marshall *et al.*, 1985). Dr. Warren and Dr. Marshall were able to culture a slow growing microaerophillic bacterium in the laboratory, by accidentally leaving agar plates containing samples from stomach biopsies in the incubator over the Easter holidays (Marshall and warren 1983) and, because it was present in nearly all individuals with active chronic gastritis, duodenal ulcer, or gastric ulcer, it was considered to be an important factor in the etiology of these diseases, but it was necessary to prove the Koch postulates by establishing a correlation between *H pylori* and disease and to fulfill the Koch’s postulates Dr. Marshall ingested a liquid culture of bacterium and immediately suffered from acute Gastritis (Marshall *et al.*, 1985) and for their pioneering work on
*Helicobacter pylori* Dr Robin Warren and Dr. Barry Marshall has been awarded the
Nobel Prize of Medicine in 2006 AD.

The morphological and physiological similarities between this new
microorganism and the Campylobacter genus, lead scientists to firstly name it as
*Campylobacter pyloridis* (Smith *et al*, 1984), which was shortly after corrected to
*Campylobacter pylori* (Marshall and Goodwin 1987). The name *Helicobacter pylori*
was conferred upon this bacterium in 1989, after important physiological
differences between this organism and other Campylobacter spp. were identified
(Goodwin *et al*, 1989). Since those early days, many advances in the understanding
of *H. pylori* particular characteristics were achieved.

Isolation of *H. pylori* was a significant scientific result, but still did not
established whether the bacteria were the cause of the inflammation with which
they were associated, or whether they occurred as a result of it. However, the
scientific field has rapidly moved forward during the last two decades of
*H. pylori* research, many advances in the understanding of *H. pylori* particular
characteristics were achieved. A great deal of the bacterium’s biochemical pathways
has been identified, the prevalence in the human population in several countries all
around the world described, and its role in certain human diseases such as gastritis
and stomach ulcer clarified.

It is more horrifying when it comes to the increasing drug resistance of this
bug to the currently existing antibiotic regimen along with undesirable side effects
compliance and contraindications in most of the patients (Ali *et al*, 2005).
Research has also allowed for the development of reliable diagnostic methods for *H. pylori* infection and suitable treatment procedures. Many questions, however, remained unclear for instance, the role of the bacteria in other diseases is still under great debate, the route of transmission between the human population as well as why merely 10% of infected people develop serious illness such as gastritis, peptic ulcer disease and gastric cancer, and why the majority (90%) of people infected with *H. pylori* suffer no symptoms related to their infection. In 1994, the National Institutes of Health (NIH) concluded that there is a strong association between *H. pylori* and peptic ulcer disease and the International Agency for Research on Cancer (IARC), part of the World Health Organization (WHO), classified *H. pylori* as a class I carcinogen.

The peculiarity of *H. pylori* has also turned the bacteria into one of the hot topics for scientists. Between 2000 and 2004, an average of 1500 papers were published annually in international peer-reviewed journals, only 600 less than *Escherichia coli* and over 1000 more than *Salmonella typhimurium*. It was also one of the first bacteria to have the genome sequenced for two different strains, J99 and 26695 (Alm *et al.*, 1999, Tomb *et al.*, 1997).

*H. pylori* was the precursor for the identification of a large number of related microorganisms inhabiting other mammals and birds gastrointestinal (GI) tract. By 2001, there were 20 formally named species comprising the genus Helicobacter (Solnick, J. V., and D. B. Schauer. 2001 ), and new *Helicobacter* species are now regularly being discovered (Won, Y. S and J. H. Yoon *et al.*, 2000). Most of the members do not normally colonize the gastric mucosa, but instead thrive in the intestinal tract and/or the liver of the host and are therefore classified as
enterohepatic (Schauer, D. B. 2001). Those inhabiting the stomach, such as *H. pylori*, are termed gastric (Danon, S. J., and A. Lee. 2001). So far, only *H. pylori* has been found to generally inhabit humans, even though *H. heilmannii* has been occasionally detected.

One of the most distinctive features of *H. pylori* is the genetic diversity it displays between clinical isolates. Such genetic diversity allows for single clinical *H. pylori* isolates to be discriminated from others by DNA fingerprinting techniques (Akopyanz *et al.*, 1992, Wang *et al.*, 1993, Marshall *et al.*, 1995, Marshall *et al.*, 1996) or sequencing of corresponding genes (a high degree of sequence divergence can be seen between orthologues (3-5%) and identical sequences are rare) (Achtman *et al.*, 1999, Owen and Xerry, 2003). In addition, *H. pylori* has been shown to have a panmictic or freely recombining population structure (Go *et al.*, 1996) and is naturally competent (Israel *et al.*, 2000). These characteristics allow for intra-strain rearrangements and horizontal transfer of foreign genetic sequences, a common occurrence in *H. pylori* isolates (Devi *et al.*, 2006).

Attempts to understand the cause and potential benefits of *H. pylori*’s genetic diversity have led to some interesting discoveries relating to the co-evolution between host and bacterium, quasi-species development, virulence determinants and eradication strategies. Recent studies summarized herein pose the question as to whether *H. pylori* may be beneficial to human health in certain circumstances and whether eradication of this organism is necessary in all cases.

Although DNA fingerprinting techniques demonstrated considerable genetic diversity between clinical isolates of *H. pylori*, it was not until the full sequences of two *H. pylori* genomes were released that the extent of its diversity was realised.
Recombinational events including the presence of insertion elements, pathogenicity islands, horizontally acquired genes (restriction recombination genes), mosaics and chromosomal rearrangements were found frequently (Tomb et al, 1997; Alm et al, 1999). It has been suggested that such diversity is a result of a lack of direct competition between strains, even when resident within different individuals within the same community (Blaser and Berg 2001). An interesting fact discovered post-genome sequencing is that there is a region in both the strains where 48% and 46% of strain-specific genes are located in J99 and 26695 respectively, this region has been termed the ‘plasticity zone’ (Alm et al, 1999). Interestingly, a novel pathogenicity marker (JHP947) has been detected within the plasticity zone (Santos et al, 2003). Many genes linked to development of gastric cancer have been assigned to the plasticity zone (Occhialini et al, 2000). Genome sequence comparisons have revealed that nearly half of the strain specific genes fall in this zone. Recently, a new type IV secretion apparatus has been located in this plasticity zone (Figure 1). (Kersulyte et al, 2003).

**Figure 1: Helicobacter pylori tfs3**

This type IV cluster is comprised of 7 genes, homologous to the vir B operon of *A. tumefaciens* carried in a 16.3kb genomic fragment called tfs3 (Figure 2).

**Figure 2: Agrobacterium tumefaciens vir B operon**
This cluster was discovered by Kersulyte *et al.*, as a result of subtractive hybridization and chromosome walking and sequence homology. They also tested conservation of this island in clinical isolates and found that full length and partially disrupted tfs3 occur in 20% and 19% of the strains respectively, from Spain, Peru, India and Japan. Although there is no correct role assigned to this cluster, it might be an unusual transposon linked to many deletion events occurring in the plasticity region that contribute to bacterial fitness in diverse host populations via exercising flexibility in gene content and gene order.

The diverse nature of *H. pylori* and the evidence of horizontal transfer of genes from other *H. pylori* isolates and bacterial species could explain the ability of this organism to persist in a changing environment and why only a subset of clinical isolates exert an adverse effect on patients.

More recently significant association reported for the genes JHP0940 and JHP0947 with gastric carcinoma, divert the attention of researchers towards the plasticity region in the hope of finding a new marker that could provide important clues in understanding the mechanism of differential expression of genes controlling the outcome of the infection. Since the plasticity region of strains J99 and 26695 possess certain characteristic of potential PAI, it is interesting to investigate the role of ORFs with hypothetical functions organized in such a PAI. Our interest is to identify genes that are expressed selectively in response to bacterium-host interaction in the gastric mucosa and that can be used as diagnostic markers for the determination of clinical outcome of the infection. Since there has been no work reported in case of Indian strains on the characteristic behavior of plasticity region
of *Helicobacter pylori*, it is interesting to study the same, taking all these points into consideration this study has been initiated with the following aims and objectives.

1. Culture and isolation of *H. pylori* isolates from gastric biopsies and characterization for known genes (cagA, 16S rRNA, and *iceA*) by PCR based assays, and its prevalence with different disease categories.

2. Computational analysis of plasticity region sequences of *H. pylori* strains J99 and ATCC 26695 and identification of important candidate genes (these sequences are available in public domain).

3. Testing of candidate genes for presence/absence or rearrangement and functional association if any in Indian isolates and histopathological analysis of the gastric biopsies obtained from the dyspeptic patients at the time of endoscopy.

4. Prevalence study of Plasticity region ORFs of *Helicobacter pylori* and its correlation with the disease status.

5. In silico analysis of *Helicobacter pylori* plasticity region ORFs and the secondary structure prediction of the ORFs from Plasticity region.

6. Cloning and Expression of Plasticity region ORFs and characterisation of the isolated recombinant protein with respect to immunological assays such as ELISA.

7. Searching for the diagnostic marker for the *Helicobacter pylori* infection through the recombinant proteins of Plasticity region ORFs by using ELISA.
The outcome of the proposed research is likely to help the health care professionals through rapid and accurate molecular diagnostics to monitor the pathologic behavior of strains and to forecast whether or not a single infecting strain will be able to produce severe pathology including cancer.
Prevalence of \( H. \) pylori in worldwide human populations.