We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Chapter 5

Helicobacter pylori—Associated Dyspepsia in Paediatrics

Mónica Roxo-Rosa, Mónica Oleastro and
Ana Isabel Lopes

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/56551

1. Introduction

Helicobacter pylori ubiquitously infects the human gastric mucosa since time immemorial, predictably before the man's diaspora out of East Africa around 58,000 years ago [1]. Colonization may have been somehow beneficial for human carriers, allowing the co-evolution of this gram-negative bacterium and its host over the centuries. Yet, at least nowadays [2], this may not be a peaceful association, with infection almost invariably causing an acute host immune response. However, in a fully adapted manner, *H. pylori* avoids recognition and, thus, clearance, by the host immune system, with both infection and the consequent gastritis persisting throughout the patients' life. The clinical outcome of this persistence is dependent on a sophisticated crosstalk between the host and the pathogen. If often asymptomatic, the *H. pylori*-associated non-ulcer dyspepsia is clearly the strongest aetiological factor for severe gastric diseases that will develop late in adult life in a minority of infected patients, *i.e.*, peptic ulcer disease, both gastric and duodenal ulcers, and gastric cancer, namely, adenocarcinoma and mucosa associated lymphoid tissue (MALT) lymphom*a (reviewed in [3])*. Peptic ulcer disease rarely occurs soon after *H. pylori* infection [4-8] that generally starts in childhood; this presumably reflects marked differences in the virulence [9-16] and/or in the susceptibility of young patients [17-19].

This chapter, focussing on the paediatric population, seeks to explore: the prevalence of *H. pylori* infection; the molecular mechanism used by *H. pylori* during colonization and infection; the role of this bacterium in the development of peptic ulcer-related organic dyspepsia; and the genetic/proteome profile of the *H. pylori*-strains associated with peptic ulcer disease.

INTECH
open science | open minds

© 2013 Roxo-Rosa et al.; licensee InTech. This is a paper distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1.1. Prevalence of infection

H. pylori is one of the most common gastrointestinal bacterial infections among humans, affecting more than 50% of the world's population [1,20]. *Infection is usually acquired during the first years of life in both developing and industrialized countries, with* intra-familial spread playing a central role in transmission of the infection [21,22]. The prevalence of *H. pylori* is markedly variable between developing and developed countries, and even among individuals living in the same country, varying according to ethnicity, place of birth and socioeconomic factors. Besides geographic area, age is also significantly and independently associated with an increase in *H. pylori* prevalence, a phenomenon known as birth cohort effect, which is a progressive reduction of the infection rate in successive birth cohorts, due to the improvements in general living conditions (reviewed in [23]). In less developed countries the infection rate reaches almost 50% in very young children and more than 90% in adults, whereas in industrialized countries *H. pylori* infects 20-50% of adults and less than 10% of children, and has been declining over time [23,24]. Indeed, the prevalence of *H. pylori* infection is *showing a decreased trend* worldwide that *is directly associated with an improvement in the socioeconomic status and hygienic conditions of the populations.*

Accordingly, *in Europe and North America, the epidemiology of H. pylori infection in children has changed in recent decades. Nowadays, low incidence rates are found in the northern and western European countries, resulting in prevalence far below 10% in children and adolescents. In contrast, the infection is still common in certain geographic areas such as southern or eastern Europe, Mexico, and certain immigrant populations from South America, Africa, most Asian countries, and first-nation (aboriginal) people in North America* [25-27]. In Portugal with the worst scenario of Europe, the prevalence of H. pylori infection is closer to the situation observed in developing countries, reaching 80% among the adult population in their early nineties, and, more recently, varying from approximately 20% in young children (less than 5 years old) to 50% in children 10 to 15 years old [28,29].

The absence of effective vaccines [30] and of efficient alternatives to antibiotics [31-34] renders difficult the worldwide prevention of *H. pylori* infection-associated diseases through massive eradication of the bacterium. The current antibiotic therapy against *H. pylori* infection fails in about 20% of the patients; depending on the therapeutic schema and strain resistance pattern, the failure rate may reach 70%. Antibiotic resistance, mainly to clarithromycin, is the major factor affecting the efficacy of standard triple therapy of *H. pylori* infection (co-administration of two antibiotics and a proton pump inhibitor or ranitidine bismuth for seven to ten days). In fact, the resistance rates to this and other second line antibiotics, such as the fluoroquinolones, are increasing in many geographical areas [34-36].

Several studies reveal a similar or higher resistance rate to clarithromycin among paediatric isolates as compared to those obtained from adults, especially in southern European countries, reflecting the recognized overuse of macrolides in children in these countries [31,34,37,38]. As an example, Portugal displays one of the highest rates of *H. pylori* primary resistance to clarithromycin in Europe, similarly high in children as among adults (≈33%) [34]. Moreover, resistance to second line antibiotics has rapidly increased over the last decade and is a matter of concern [31-34]. This places the research on disease-specific bacterial biomarkers and their

http://dx.doi.org/10.5772/56551

associated molecular mechanisms as a top priority to define disease-risk and to target *H. pylori* eradication in high-risk individuals. Ultimately, it may provide novel bacterial and/or host's therapeutic or vaccine targets.

1.2. Molecular mechanisms of *H. pylori* colonization and infection

1.2.1. Acid resistance and motility

In a fully adapted manner, during colonization and persistence, this neutralophile bacterium resists gastric acidity mainly through its urease activity. Its urease enzyme, a Ni^{2+}-containing dodecameric protein of approximately 1100 kDa, composed of 12 small subunits, UreA (27 kDa), and 12 large subunits, UreB (62 kDa), catalyzes the hydrolysis of urea into ammonia and carbon dioxide, buffering both the bacteria cytoplasm and periplasm [39]. Accounting for 5-10% of the total protein content, urease is one of the most abundant proteins in the *H. pylori* proteome [16,30]. Probably due to the toxicity of ammonia, urease activity is known to be dependent on low pH and/or Ni^{2+} concentration conditions [39,40], being essential for bacteria survival only under acidic conditions. In the early stages of colonization, *H. pylori* seeks parts of the stomach with higher pH, such as the antrum (the distal part of the stomach). Indeed, this bacterium uses the pH gradient as chemotactic signal to achieve regions of neutral pH, since its spatial orientation is lost in the absence of the mucus pH gradient [41]. Thus, the acid-producing parietal cells may protect the corpus region from initial invasion.

Efficient colonization of the gastric niche by *H. pylori* is also dependent on how fast it escapes from the lumen of the stomach and reaches the mucus layer, avoiding elimination by gastric peristalsis [42]. Its helical shape and the two to six polar, sheathed flagella provide swimming abilities. According to a longstanding theory [43], the helical shape allows *H. pylori* to have a corkscrew motion which, although not being essential for motility, enhances its ability to swim through the viscous mucus layer. The machinery that gives rise to the spiral shape of this bacterium remains largely unknown, but seems dependent on the coordinated action of multiple proteins in a shape-generating pathway that leads to the relaxation of the peptidoglycan crosslinking [44]. Flagella are, however, essential for *H. pylori* motility. Indeed, aflagellated strains (obtained by elimination of both *flaA* and *flaB*, genes encoding the two major components of flagellar filament, flagellins A and B) [45], as well as strains presenting non-functional flagella (in knockout *motB* models lacking the gene that encodes the MotB flagellar motor protein), are non-motile [42]. Such mutants are able to establish only transient colonization in animal models [42,46]. Moreover, lower-motility strains are long known to induce *in vitro* reduced inflammation levels, when compared to higher motility strains [47]. Once in the mucus layer of the stomach, *H. pylori* resides here thereafter, either freely swimming [43] or attached to host's extracellular mucins [41], getting closer to the host's gastric epithelial surface whenever necessary. Occasionally *H. pylori* also can be internalized, entering the gastric epithelial cells [48]. Invasion beyond the epithelial layer is, however, is a rare event.

1.2.2. Bacterial Adherence

In the human stomach, the vast majority of *H. pylori* cells exist in their motile form within the mucus layer lining; only a small portion (≈30%) are adherent to the surfaces of epithelial cells [41]. Nevertheless, adherence to the gastric epithelium is important for the ability of *H. pylori* to cause disease because this intimate attachment facilitates: 1) persistence, by preventing the bacteria from being eliminated from the stomach through mucus turnover and gastric peristalsis, and also by enabling the bacteria to replicate; 2) evasion from the human immune system; 3) efficient delivery of the bacterial toxic proteins; and 4) acquiring nutrients released from the damaged host cells.

H. pylori expresses a multitude of different adhesins. Best characterized is the blood group antigen-binding adhesin (BabA), a ligand of ABO (of the blood group system) Lewis b (Le^b) antigens [49]. Sequence analyses reveals the existence of two allelic variants of *babA*, the *babA1* and *babA2* alleles, which are identical except for a 10 base pair insertion that results in a translational initiation codon present in *babA2* but absent in *babA1*, and of a highly homologous gene, *babB*. Of these, only *babA2* allele encodes a functional Le^b adhesin [49]. BabA is not likely to be essential for the colonization; BabA-expressing strains are no different in this step compared to BabA-non-expressing strains [50]. BabA, being an adhesin, however likely plays an important role in the induction of host inflammatory response. Indeed, *babA2* allele is clinically important, namely in a *vacA*/*cagA*-positive genetic background (two additional important virulence factors discussed in section 1.2.3. of this chapter), which is associated with peptic ulcer disease and gastric cancer [51]. BabA-expressing strains induces change in the glycosylation pattern of the gastric mucosa of humans and animal models [50].

The second best characterized *H. pylori* adhesin is the sialic acid binding adhesin (SabA) which mediates attachment to the inflammation-associated (sialylated $Lewis^x$ and $Lewis^a$) antigens [52]. In fact, gastric tissue inflammation and malignant transformation promote synthesis of sialylated glycoconjugates, which are rare in healthy human stomachs [52, 53]. Accordingly, high levels of sialylated glycoconjugates are found in *H. pylori* infected persons; these decrease after eradication of the infection and resolution of the gastritis [54]. *H. pylori* can agglutinate erythrocytes and neutrophils *in vitro*. The SabA adhesin is the hemagglutinin of *H. pylori* and allows bacterial adherence to blood cells; this may result in systemic dissemination of the pathogen [55]. Moreover, the binding of SabA to sialic acid carries neutrophil receptors, essential for the nonopsonic activation of human neutrophils [56]. Neutrophils play a major role in the epithelium injury, since these cells have direct toxic effects on the epithelial cells, through the induction of an oxidative burst, with the release of reactive oxygen and nitrogen species. Thus, the neutrophil activating capacity of SabA makes this protein an additional virulence factor that is important in the pathogenesis of *H. pylori* infection.

The outer membrane inflammatory protein (OipA), a member of the Hop family of proteins, is another *H. pylori* membrane protein with an active role in bacterial adherence, for which no host receptors are known. Its encoding gene (*oipA*) is present in all *H. pylori* strains but it is only expressed in those presenting the *oipA* "on" (*i.e.*, functional) genotype. This is regulated by slipped-strand mispairing, depending on the number of CT repeats in the 5′ region of the gene [57]. OipA expression by *H. pylori* is associated with high bacterial densities, severe

neutrophil infiltration and, ultimately, with peptic ulceration and gastric cancer [58,59]. Supporting the later association, the inactivation of *oipA* results in a reduced nuclear translocation of β-catenin, a known factor involved in the transcriptional up-regulation of genes implicated in carcinogenesis [59].

1.2.3. Delivery and virulence factors

After adherence, *H. pylori* delivers its virulence factors into the cytoplasm of the host's cells by using a type IV secretion system (T4SS) and/or outer membrane vesicles. The genes encoding the components of the T4SS, which is a syringe-like pilus protruding from the bacterial surface used to inject virulence factors in host target cells' cytoplasm, are located in the *cag* pathogenicity island (PAI) [60]. This is an approximately 40 kpb chromosomal insertion that is thought to have been incorporated into the *H. pylori* genome by horizontal transfer from an unknown source [61]. As a result, strains are heterogenic regarding the presence of this chromosomal region, varying between those that contain the intact *cag* PAI to those that completely lack it. For those lacking an intact T4SS, the delivery of their virulence factors is totally dependent on the secretion of outer membrane vesicles with a still poorly known content; these are endocytosed by the host epithelial cells (reviewed in [62]).

Encoded by *cytotoxin associated gene A*, one of the 32 genes of the *cag* PAI region, CagA is perhaps the most extensively studied translocated protein, the only known effector protein injected by the T4SS. Once injected into cytoplasm of target cells, CagA interferes with several host cell signalling cascades, ultimately inducing abnormal proliferation, cytoskeleton rearrangements and inflammation through the release of cytokines, such as interleukin-1β (IL-1β), IL-8 and tumour necrosis factor-α (TNF-α) (reviewed in [63]). There are two types of clinical isolates regarding CagA: those producing this protein (*cagA*-positive *H. pylori* strains), and the CagA-nonproducing strains (*cagA*-negative *H. pylori* strains). The *cagA*-positive strains are considerably more virulent [60]. In Western countries, individuals carriers of *cagA*-positive strains are thus at higher risk of peptic ulcer disease and/or gastric cancer [64,65], leading to the classification of CagA as a bacterium-derived oncogenic protein [66]. In a not fully undisclosed manner, the virulence of the *cagA*-positive strains is associated with the number and the type of the phosphorylation motifs of the C-terminal variable region of the protein. Of these motifs, defined as EPIYA (Glu-Pro-Ile-Tyr-Ala) A, B, C and D according to different flanking amino acids, CagA protein nearly always possesses EIPYA-A and B segments, followed by none, one, two or three C segments in Western-strains or a D segment in strains of East Asian countries. The East Asian-type of CagA (ABD) is known to be more carcinogenic than the Western-type; within the latter, the variants possessing multiple EPIYA-C motifs (ABCC or ABCCC) are more virulent compared with those with a single segment (ABC) (reviewed in [63]). Moreover, the association of CagA expression levels with polymorphisms in the *cagA* promoter region may further contribute to differences in virulence among *cagA*-positive strains and different disease-associated risks [67]. The virulence of the strain must also be dependent on additional bacterial factors, since in East Asia most strains are *cagA*-positive irrespective of the patient disease.

The vacuolating toxin (VacA), another important virulence factor (reviewed in [68]), is synthesized as a pro-toxin of ≈140 kDa, which contains a *N*-terminal signal sequence, a passenger domain and *C*-terminal autotransporter domain. The passenger toxin domain (≈88 kDa) is cleaved and processed at some point during its secretion into the extracellular milieu through the autotransporter that functions as a type V secretion system. This 88 kDa toxin is further proteolytically cleaved, creating a *N*-terminal fragment of ≈33 kDa (p33) and a *C*-terminal fragment of ≈55 kDa (p55) that remain non-covalently associated. Required for the cytotoxic activity, the p33 subunit is postulated to be involved in the formation of anionic membrane channels, while p55 subunit seems to mediate VacA binding to host cells (reviewed in [68]). These functions are, however, highly dependent of the tridimensional structure of both subunits [69,70]. Once secreted by the bacterial cells, VacA triggers various responses in the host, resulting in the cellular vacuolation, pore formation in the cell membrane, disruption of endosomal/lysosomal structure and function, apoptosis by toxin trafficking to mitochondria, and immunomodulation [71-73]. Virtually all *H. pylori* strains have a functional VacA. However, the amount of toxin produced is related to the allelic variation of the encoding gene, especially in its signal and middle regions (reviewed in [74]). Therefore, an association between particular *vacA* allele types and peptic ulcer disease has been reported worldwide..

The ancient association between *H. pylori* and the modern humans [1,20] has determined the abnormal high diversity in both their genetic background and the virulence observed among strains. This generates complex scenario that creates difficulty in understanding the contribution of each individual factor. Nevertheless, *H. pylori* strains presenting the association of these two virulence factors, *i.e.*, *cagA*-positive and *vacA*-toxigenic alleles, are considered to be more virulent and thus more associated with severe organic dyspepsia inducers of a high production of proinflammatory cytokines in the gastric mucosa and thus more severe non-ulcer dyspepsia. Even so, these virulence factors do not appear to determine the overall pattern of gastroduodenal disease and a complex interplay between host bacterial factors and environment seems to be involved in the development of gastric pathology [75].

1.2.4. Evasion

Upon colonization, *H. pylori*-infected patients experience a strong and complex immune response in the gastric mucosa, both at the humoral and cellular levels; despite this response, nevertheless the infection fails to clear. Therefore, in the absence of effective treatment, infection becomes chronic, persists, and contributes to the immunopathology. The persistence of infection throughout the life of its host is guaranteed by a set of molecular mechanisms used by *H. pylori* to constantly evade the host immune response (reviewed in [76]). Bacterial mimicry and genetic diversity play a central role in such successful strategies.

Mimicking the cell surfaces of the host, the lipopolysaccharide of the *H. pylori* cell wall is relatively anergic compared to other gram-negative bacteria. In fact, the variable part of the *O*-antigen chain of *H. pylori* lipopolysaccharide is composed of host-related Lewis antigens, making it unrecognizable to the host immune system [77]. Therefore, this pathogen not only binds to human Lewis antigens through BabA and SabA, but it also expresses Lewis-like antigens facilitating the escape from the host immune system. Another ingenious camouflage

http://dx.doi.org/10.5772/56551

used by *H. pylori* is the expression of proteins at its surface, which specifically bind to host-secreted proteins, *e.g.*, bacterial plasminogen-binding proteins (PgbA and PgbB). This allows the bacterium to be coated with host proteins [78]. *H. pylori* also avoids immune recognition through the *in vitro* and *in vivo* impairment of the expression of host's specific heat shock proteins, thus, inactivating both the innate and adaptive immune response [79].

Allelic diversification of its virulence factors encoding genes allows *H. pylori* to occupy different microenvironments within the human stomach and to adapt to the varying conditions in the niche over time. This is even more efficient, considering that the expression of different variants of those genes may switch through mechanisms of phase variation. Indeed, several *in vitro* studies have demonstrated that *H. pylori* Lewis-like antigens can undergo phase variation (reviewed in [76]). Moreover, in animal models, persistent infection leads to the loss of expression of *babA*. This occurs either by phase variation switching between an "on" and an "off" status in a manner similar to that described for *oipA* (see section 1.2.2 of this chapter), or by nonreciprocal gene conversion of *babA* to *babB* [80].

The existence of a small subpopulation of *H. pylori* within gastric epithelial cells (as briefly discussed in section 1.2.1 of this chapter) may represent a sanctuary site that protects bacteria against immune clearance [48].

1.3. Peptic Ulcer — Related organic dyspepsia in paediatrics, a rare event

Although rarely associated with severe forms of organic dyspepsia (namely peptic ulcer disease) in the paediatric age group, *H. pylori* is clearly linked with acute gastric inflammation in childhood and occurs frequently in children with dyspepsia [81]. This important association gains relevance when considering that: a) gastric colonization by *H. pylori* occurring in childhood and the consequent inflammation continues for life if left untreated; and b) *this* lifelong persistence of inflammation after decades of infection is the main etiology for peptic ulceration and/or cancer in adulthood. Moreover, some studies suggest a possible impact of *H. pylori*-associated dyspepsia on anthropometry, as children with dyspepsia and *H. pylori* infection are shorter and lighter than children with similar symptoms but no infection [82,83].

Evidence for the importance of *H. pylori* infection as a factor for dyspepsia in childhood comes from *H. pylori* eradication resulting in a significant long-term improvement of dyspeptic symptoms [84]. The symptoms of *H. pylori*-associated paediatric dyspepsia however do not differ from those of non-infected dyspeptic children [85], raising questions about which approach should be adopted in children with dyspepsia, in terms of *H. pylori* testing. According to current guidelines for the management of *H. pylori* infection at pediatric age [86,87], the primary goal of diagnostic interventions should be to determine the cause of the presenting gastrointestinal symptoms ("scope and treat" strategy) and not just the presence of *H. pylori* infection ("test and treat" strategy). Indeed, recurrent abdominal pain is not an indication for a "test and treat" strategy concerning *H. pylori* infection in children, as evidence regarding the association with *H. pylori* infection has been so far inconclusive (even in the presence of peptic ulcer). Indeed, several studies using different noninvasive tests for *H pylori* infection compared the prevalence of positive results in children with recurrent abdominal pain and controls and found no significant difference in infection rates between cases and controls [88,89]. On the

other hand, pediatric studies are limited by the lack of a clear definition for recurrent abdominal pain or by the use of nonspecific criteria for the diagnosis of chronic abdominal pain [90]. Nevertheless, in patients with persistent abdominal pain (after exclusion of other causes, such as lactose intolerance, giardiasis, celiac disease, *inflammatory bowel disease*, among others) and/or severe upper abdominal symptoms (namely suggesting peptic ulcer disease, such as nocturnal pain), upper endoscopy with biopsy should be performed (diagnostic investigation of choice). Furthermore, testing for *H. pylori* in children/adolescents should be considered if there is a family history of gastric cancer and in children/adolescents with refractory iron deficiency anemia, when no other cause is found. In these settings, when upper endoscopy is performed, the presence of *H. pylori* should be systematically sought through histological examination and, whenever feasible, culture and antibiotic susceptibility testing; treatment should be offered in the presence of *H. pylori* positivity. Population screening for *H. pylori* in asymptomatic children to prevent gastric cancer is not warranted. Although ^{13}C-urea breath testing is a validated noninvasive diagnostic test for *H. pylori* infection in children, a "test and treat" strategy including this tool should not be indiscriminately adopted in clinical practice at this age group, considering the fact that this test merely identifies *H. pylori* presence but not necessarily the causality of symptoms. Noninvasive tests for *H.pylori* include different methods for the detection of bacterial antigens in stool, detection of antibodies (IgG, IgA) against *H. pylori* in serum, urine, and oral samples, and the ^{13}C-UBT. In the paediatric group, both fecal antigens determination and respiratory test are reliable to determine whether *H pylori* has been eradicated or not after antibiotic treatment, while tests based on the detection of antibodies against *H pylori* are considered not reliable for use in the clinical setting, but may be useful in epidemiological studies [91].

Therefore, *H. pylori infection in childhood differs from adults not only in terms of the prevalence of the infection and a higher rate of antibiotic resistance, but also with respect to the complication rate, age-specific problems with diagnostic tests and drugs, and the near-absence of gastric malignancies* [92]. Nevertheless, H. pylori-associated peptic ulcer disease may also occur shortly after infection in childhood [4-8]. This rare event may be due to more virulent strains [9-16], and/or more predisposed subjects [17-19]. The two forms of H. pylori-associated peptic ulcers, i.e., gastric ulcer and duodenal ulcers, are divergent in prevalence and physiopathology, but both cause considerable patients' morbidity entailing high annual costs of treatment [93].

1.3.1. Prevalence of H. pylori-associated gastric and duodenal ulcers in childhood

In general, about 10-15% of the H. pylori infected patients suffer from duodenal ulcer disease and 2-5% with gastric ulcer and/or gastric cancer late in the adulthood [3]. Population-based studies in patients with organic dyspepsia suggest that peptic ulcer disease related to H. pylori infection is decreasing in prevalence in Western countries, along with a decrease in the prevalence of infection [94]. The former should be a direct consequence of the second; with improved living standards, cohorts of children became progressively less likely to acquire the organism and thus suffer from H. pylori-associated diseases. Nevertheless, specific populations such as immigrants and rural communities may have a high prevalence of infection and peptic ulcer disease; these individuals should be separately reviewed even in areas where the general prevalence of H. pylori infection has declined below 15% [95]. Despite changing

prevalence trends, H. pylori-induced gastritis causing mucosal ulceration either in the stomach (gastric ulcer) or the proximal duodenum (duodenal ulcer) is a relatively uncommon event in children, compared with adults [4-8]. In fact, during childhood, H. pylori is associated with predominant antral gastritis or with pangastritis [96]. *In children, few studies have yet investigated the actual trend of H. pylori prevalence in peptic ulcer disease* [5-8] and the available data are more difficult to interpret, considering that the rates of peptic ulcer diagnosis depend also on the clinical setting (endoscopy versus outpatient clinic or hospital admissions) [97,98]. For example, in Italy the detection rate of ulcer disease was 7.8% out of an average of 180 paediatric gastrointestinal endoscopies performed each year [99]. Similarly, a retrospective review (from 1998 to 2006) showed that 43 (6.9%) out of 619 Chinese children who underwent upper endoscopy for investigation of upper gastrointestinal symptoms had peptic ulcer [7]; and another retrospective study (from 2003 to 2006) have also reported a high incidence of peptic ulcer (6.8%) in Israeli children submitted to upper endoscopy [100]. In Canada, however, the approximate incidence of peptic ulcer was 1 case per 2,500 hospital admissions [92]. In a recent large European multicenter study, including 1233 symptomatic children with H. pylori infection, peptic ulcer disease was diagnosed in less than 5% of children younger than 12 years of age and in ≈10% of teenagers [8]. Interestingly, other studies indicate a higher association of H. pylori with peptic ulcer in adolescents than in younger children [100,101]. But, the prevalence of H. pylori-positive ulcers in children also differs between countries and this is not completely explained by the prevalence of the infection in the population studied. This is easily demonstrated from data collected from January 2001 to December 2002 on 518 children from the paediatric European register for treatment of H. pylori [5]. At endoscopy, 454 of those patients had H. pylori-associated gastritis and 64 had a peptic ulcer (12.3%). This series also included children from Russia, who had a significantly higher prevalence of peptic ulcer (35%) compared to that of the remainder of European children (6.7%) [6]. In another report, school-aged children with chronic abdominal complaints living in the rural area of Russia had a high prevalence rate of H. pylori infection (80%) and also of peptic ulcer disease (24%) [102].

In adults, the prevalence of *H. pylori* infection is higher than 95% in duodenal ulcer cases and around 60% to 80% in gastric ulcer cases [103]. This scenario is similar in children i.e. when *H. pylori*-associated ulcers occur in children, duodenal ulceration is much more frequently identified than gastric ulcers [104]. In fact, pooled analysis of early reports (from 1983 to 1994) has demonstrated that the prevalence of *H. pylori* in children with duodenal ulcer was relatively higher (ranging from 33% to 100%, with a median value of 92%), compared with children with gastric ulcer (ranging from 11% to 75%, with a median value of 25%) [104]. A more recent retrospective study (from 1995 to 2001) from Japan confirmed a very high prevalence of *H. pylori* in antral gastritis and duodenal ulcer (98.5% and 83%, respectively), also identifying *H. pylori* as a risk factor for the development of gastric ulcer although with a lower prevalence of infection (less than 50%) [101]. Finally, in a Chinese study, it was reported that among 43 Chinese children suffering with peptic ulcer disease, 37 had duodenal ulcer, of which 21 were *H. pylori* positive, while only six had gastric ulcer, of which only two were positive for the infection [7]. In summary, *H. pylori* infection is much more associated to duodenal ulcer than to gastric ulcer, in both children and adults.

The causative role of *H. pylori* in gastric ulcers in children and adolescents is, therefore, less certain when compared to adults, possibly reflecting the fact that a large proportion of gastric ulcers are secondary in nature in children. Characteristically, in children younger than 10 years of age, peptic ulcers are usually due to noxious agents (such as corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs)) or occur after major stresses (such as burns, trauma, and systemic illness). In these settings, upper gastrointestinal tract haemorrhage, vomiting and perforation are frequent presenting features. The ulcers tend not to recur after healing. In older children and adolescents, the clinical presentation and natural history of peptic ulcers are similar to that observed in adults, presenting as epigastric and nocturnal abdominal pain and being usually associated with *H. pylori* infection [8,100,101]. In this setting, even though the acute ulcer is likely to heal, the natural history is for ulcer recurrence. Moreover, the complication rate of peptic ulceration is important. The estimated incidence of peptic ulcer bleeding in the US paediatric population also has ranged from 0.5 to 4.4/100,000 individuals in 2008 [105].

These and other differences explain why some of the recommendations for adults may not apply in children [87,96]. Few randomized, placebo-controlled treatment trials are available in children for the different outcomes (gastritis or peptic ulcer), and often consist of only small numbers of cases [86,106]. Clearly in children as in adults, successful eradication of *H. pylori* markedly reduces the risk of ulcer recurrence [107-110]. Thus, there is general consensus worldwide to treat *H. pylori* infection when there is endoscopic evidence of peptic ulceration. Triple therapy is the treatment of choice in children for endoscopically proven duodenal ulcer and histologically proven *H. pylori* antral gastritis [91, 96, 111, 112].

1.3.2. Differences in the physiopathology of H. pylori-associated gastric and duodenal ulcers

Peptic ulceration is a multifactorial disease ultimately explained by disequilibrium between aggressive injurious factors and defensive gastroduodenal mucosa-protective factors, which raises the vulnerability of this mucosa to luminal secretions. *H. pylori* infection is considered the major causative factor for peptic ulceration. Nevertheless, there are other injurious mechanisms jeopardizing the mucosal integrity: some viral infections (*e.g.* cytomegalovirus and herpes simplex); drug-induced injury, particularly acetylsalicylic acid, NSAIDs and chemotherapy; vascular disorders interfering with perfusion; major stresses; and syndromes in which a marked overproduction of gastric acid occurs, as is the case of the Zollinger-Ellison syndrome and, more commonly in children, antral G cell hyperplasia (also referred to as pseudo-Zollinger Ellison syndrome) [113].

Although differing in their pathogenesis, both *H. pylori*-associated duodenal ulcers and gastric ulcers are intimately related to changes in the acid production by the gastric mucosa [113, 114]. Indeed, *H. pylori* infection can result in increased, decreased or no overall change in the level of gastric acid secretion. Duodenal ulcers arise on a background of *H. pylori*–induced antral-predominant gastritis with sparing of the oxyntic mucosa, resulting in hypergastrinemia and consequent high levels of acid production from the healthy gastric corpus following meal or hormonal stimulation. In response to the excessive acid secretion, the duodenum develops gastric metaplasia. This, unlike the normal duodenal mucosa, can be colonized by

http://dx.doi.org/10.5772/56551

H. pylori with consequent inflammation and ulceration [113]. Eradication of the *H. pylori* infection corrects the hypergastrinemia and decreases the basal acid secretory rate, heals any peptic ulcer and ameliorates any symptoms of gastroesophageal reflux [115]. Conversely, gastric ulcers are associated with *H. pylori*–induced pan- or corpus-predominant gastritis, resulting in multifocal atrophy of acid-secreting mucosa and reduced acid secretion. These ulcers usually arise at the junction of the antral and corpus mucosa, an area of intense inflammation [113]. Thus, the non-acidophilic nature of *H. pylori* (see section 1.2.1) explains how those with low acid secretory capacity are more susceptible to spread of infection through the corpus mucosa and to gastric ulceration. With their somewhat common pathobiology, gastric ulcer disease precedes the development of gastric cancer [93,116,117]. Gastric and duodenal ulcers have marked differences in their basis, placing them on opposite ends of disease spectrum. *H. pylori*-induced duodenal ulcer conveys a lower risk of developing a gastric cancer [117]. But, what makes it possible for *H. pylori* to be involved in both ends of disease spectrum? Although the mechanisms are unclear, the infecting strain itself may play a crucial role on the diverging point of this disease spectrum [16,93]. Moreover, the similarity between the phenotype of gastric ulcer and gastric cancer raises questions about the carcinogenic potential of the associated *H. pylori* strains [93]. Certainly the etiology for *H. pylori*-associated peptic ulcer in adults depends on the complex interplay of gastritis phenotype and of progressive physiological gastro-duodenal alterations through childhood until adulthood, a result of environmental factors, bacterial virulence factors and host genetic background.

Despite epidemiological evidence that infection during childhood is seldom associated with peptic ulceration or gastric atrophy, the mechanisms underlying differences in histopathology and clinical expression of *H. pylori* infection when compared to the adult, are still poorly identified. Theoretically, such differences might be explained by qualitative and/or quantitative differences in induced immune response, possibly age-related. Indeed, adults exhibit a predominantly neutrophil infiltrate, whereas *H. pylori*-associated gastritis in children is usually mild and superficial with a predominantly mononuclear infiltrate, a paucity of neutrophils and a higher degree of lymphoid follicular hyperplasia [118]. Therefore, different immunopathology and different patterns of cytokine expression would be anticipated for children when compared to adults [18]. There may be differences in adaptive component of gastric mucosa immune response in children compared to the adult host; a clear Th1 response has not always been demonstrated for young patients. The lower gastritis scores in children may also be a reflection of such a skewed Th1/Th2 balance, which may result in their lower risk for developing ulcer disease [18, 19]. These findings could indicate that the host humoral and cellular responses differ depending on the age at which the gastric infection is first acquired and might explain the varying rates of disease outcomes that are evident in different parts of the world. Nevertheless, higher anti-*H. pylori* IgG antibody titres occur in paediatric patients with duodenal ulcer compared to those without ulceration, suggesting that local humoral immune responses contribute to the development of peptic ulceration in these young patients [102,119,120]. This is not surprising, given the fact that the more severe inflammation, the greater the chance of ulcer formation [121] with increased IgG production leading to mucosal damage similar to an Arthus reaction [2].

1.3.3. Endoscopic features

Endoscopy is the only method to accurately diagnose peptic ulceration in children [87,122]. A nodular mucosa in the gastric antrum or duodenal bulb and/or gastric or duodenal erosions or ulcerations are specific (but not sensitive) features, suggesting active *H. pylori* infection. For those with suspected infection, biopsies should be obtained for histopathology, as well as complementary tests for detection of *H. pylori* including rapid urease test, histopathology with Giemsa stain and, if available, culture. The rationale for the recommendation to perform more than one diagnostic test is based on their sensitivity results in children, which range from 66% to 100% for histology and from 75% to 100% for rapid urease tests [91]. In all paediatric age groups, for patients receiving therapy with a proton pump inhibitor, biopsies should be performed on the body and cardia (and, possibly, transition zones) of the stomach as well as from the antrum to reduce the chances of false-negative results. Follow-up endoscopy is rarely necessary, except in the setting of peptic ulceration associated with complications (such as haemorrhage or perforation).

1.3.4. Host susceptibility

The multifactorial nature of peptic ulcer disease reflects its dependence on the patients' genetic susceptibility and habits (alcohol and/or non-steroid anti-inflammatory drug consumption, diet, smoking and stress) [20]. Paediatric peptic ulcer disease is significantly more frequent in boys than in girls (63.6% *versus* 36.4%, $p<0.025$) [32]. Although female hormones may have a protective role against developing peptic ulcerations [123], the true nature of this susceptibility of the male gender remains unclear.

Mucins, glycoproteins secreted by the gastric mucosa, form a gel layer that is essential to maintain a stable neutral pH adjacent to epithelium. This mucus barrier affords protection from attack by acid-pepsin and other luminal noxious agents [124]. *H. pylori* has a complex relationship with different gastric mucins' subtypes. Infected children for example have a decreased mucin in their gastric mucosa presumably weakening this important defense barrier [125]. The highly diverse carbohydrate structure of the gastric mucins, functioning as binding sites for *H. pylori*, should also play a role in the outcome of infection, with genetic and epigenetic changes in the mucin molecules influencing the susceptibility of the patient for *H. pylori*-associated peptic ulcer disease. Recently, it was shown that *H. pylori*-infected children presented a normal pattern of expression and glycosylation of mucin 5AC (MUC5AC) in the surface mucous cells, and MUC6 in the gland mucous cells, contrasting with the aberrant expression of MUC6 and MUC2 found in infected adults. Additionally, it was shown that the pattern of Lewis blood group antigens in the surface epithelium of children was significantly correlated with *H. pylori* load, however no correlation with gastritis, nodularity, and gastric or duodenal ulcer was found [17].

In children and teenagers, as in adults, the severity of antral inflammation strongly correlates with the risk of duodenal ulcer disease. Among the host factors, polymorphisms in cytokines encoding genes, or in their promoters, that affect cytokine transcription, are good risk candidates. Indeed, polymorphisms in the IL-1 gene cluster play an important role in modulating the risk for *H. pylori*-induced hypochlorhydria and, thus, for gastric ulceration and cancer. The

http://dx.doi.org/10.5772/56551

IL-1 cluster, located on chromosome 2q12-22 region, includes the genes *IL-1A*, *IL-1B* and *IL-1RN* that code for the proinflammatory cytokines IL-1α, IL-1β and their endogenous receptor antagonist IL-1RA, respectively. The less common alleles of *IL-1B*, *i.e.*, *IL-1B-31C* and *IL-1B-511T* (representing, respectively, T-C and C-T transitions at positions 31 and 511 of the *IL-1B* promoter) are associated with a higher risk of hypochlorhydria [116]. This association can be explained considering that such polymorphisms lead to increased IL-1β expression/secretion that, upon *H. pylori*-infection, amplifies the host inflammatory response. Also the less common allele of IL-1RA, *i.e.*, the IL-1RN*2 (representing one of the five known 86 base pair tandem repeat polymorphisms in intron 2) is associated with a higher risk of gastric cancer in adults [116]. The risk is potentiated when in association with infection by *cagA*/*vacA*-positive *H. pylori* strains, highlighting the interplay between host and bacterial factors that seems to be involved in the development of gastric pathology [126]. Children presenting the IL1RN*2 allele and infected by *cagA*-positive *H. pylori* strains are at higher risk of duodenal ulceration, emphasizing differences in the physiopathology of the disease between adult and paediatric patients [124]. Also at higher risk of developing duodenal ulcer are children presenting the transition G-A at position 238 of the TNF-α coding gene when infected by *iceA1*-positive *H. pylori* strains [127].

Other putative host risk factors for *H. pylori*-severe gastroduodenal diseases are the polymorphisms in the genes coding for Toll-like receptors (TLRs) that might influence the innate and adaptive immune response to the infection. Indeed, the presence of the TLR4 allele in combination with infection by *cagA*-positive strains, leads to increased gastric levels of IL-8 and IL-10 [128].

1.4. Molecular profile of ulcerogenic paediatric *H. pylori* strains

The co-evolution between *H. pylori* and the modern humans has determined the extremely high diversity of the bacterium in both its genetic background and virulence. Thus, it is likely that bacterial determinants may influence the clinical outcome, an association that is well established for *cagA*, *vacA* and *babA* genes (see sections 1.2.2 and 1.2.3 of this chapter). Nevertheless, this topic is far from being fully clarified, and the identification of other factors responsible for the enhanced virulence of the bacteria leading to the development of more severe diseases remains pertinent. For that purpose, the study of *H. pylori* strains isolated in specific clinical situations, such as the paediatric peptic ulcer disease, can be useful. Indeed, *H. pylori* paediatric infection may be regarded as a privileged natural study model of the interaction of this bacterium with human host, as the child is usually not exposed to injurious factors as is the adult and represents a different stage of *H. pylori* infection in a immunologically maturing host. Comparative genomic studies of the rare paediatric ulcerogenic *H. pylori* strains and of the non-ulcerogenic strains show a distinctive genotype virulence pattern, suggesting a potential pathogenic role for new markers [9-15]. Two putative virulence determinants are associated with peptic ulceration, mostly duodenal ulcer, in children and with other *H. pylori*-virulence factors: j*hp0562*, involved in lipopolysaccharide biosynthesis and in the regulation of Lewis antigen expression [13]; and *homB*, a putative outer membrane protein, involved in bacterial adherence [9,11,12,14,15]. HomB contributes to the proinflammatory

characteristics of *H. pylori*. Strains that are also positive for both *homB* and *jhp562* are related to a higher risk of paediatric peptic ulcer disease. Thus, it is likely that these new markers acting together with the well-established virulence markers will promote a more severe antral inflammation, a phenomenon strongly associated with duodenal ulceration.

Other pathogenic genes interact synergistically to induce peptic ulcer in young patients. There is no gene or protein that acts alone to establish the virulence of *H. pylori* [74]. Accordingly, we investigated further virulence-associated genes by comparing the proteome of a group of genetically/epidemiologically-unlinked *H. pylori* strains, all isolated from Portuguese children, half suffering with peptic ulcer disease, and the other presenting only active gastritis [16]. Despite the typical proteome profile of all the *H. pylori* strains grown under the same laboratorial conditions [129], the ulcerogenic paediatric *H. pylori* strains presented differences suggestive of higher motility, better antioxidant defences and a metabolism favouring the biosynthesis of aromatic amino acids. As already mentioned in this chapter (see section 1.2.1 of this chapter) motility is a long known virulence-related trait [46], with lower-motility associated reduced inflammation levels [47] and with non-motile strains unable to establish a robust infection [42,45,46]. Moreover, it was more recently shown that higher motility enhances *H. pylori* density and inflammatory response in dyspeptic patients [130].

The differences in the abundance of antioxidant proteins observed between paediatric ulcerogenic and non-ulcerogenic strains may be important in conferring resistance to inflammation; the enzymes involved in key steps in the metabolism of glucose, amino acids and urea may be advantageous to respond to fluctuations of nutrients [16].

Additionally, by comparing the duodenal ulcer-associated paediatric strains with the one studied strain associated with gastric ulcer, we observed differences on the abundance of proteins associated with acid resistance and motility. These suggest that the former are better prepared to survive to the abnormal low levels of pH observed in duodenal ulceration, in contrast to the gastric ulcer strain which is a better swimmer, supporting the proximal spread of infection characteristic of this disease [16]. Overall, our data supports the idea that the infecting strain may be determinant in the divergence between duodenal and gastric ulcer [93].

2. Conclusions

The prevalence of *H. pylori* infection remains high worldwide despite a progressive decline over time, attributed to improved overall living conditions and hygiene. Although often asymptomatic, most infected patients suffer from persistent non-ulcer dyspepsia that, usually later in adulthood, may further progress to more severe conditions. The most common severe complication *H. pylori* is duodenal ulcer, affecting 10 to 15% of the infected adults. Although less frequent, 2 to 5% of the infected adults with non-ulcer dyspepsia progress to gastric ulceration and some ultimately to gastric cancer. These two forms of peptic ulcer-related (organic) dyspepsia differ in prevalence and physiopathology; those suffering with duodenal ulcer are at low risk of developing a gastric ulcer/gastric cancer. The onset of peptic ulcers in childhood is a rare event that may occur shortly after infection, suggesting more virulent *H.*

http://dx.doi.org/10.5772/56551

pylori strains and more susceptible young patients. *H. pylori*-associated paediatric peptic ulcer disease is, therefore, a privileged natural study model to search for ulcerogenic-specific bacterial biomarkers and implicated molecular mechanisms, a required step to better address this important public health problem. This includes enhanced virulence of the paediatric ulcerogenic *H. pylori* strains that also may have a natural ability to better adapt to the hostility of their niche.

Acknowledgements

This work was supported by BNP Paribas patronage and a research Grant from the Sociedade Portuguesa de Gastrenterologia.

Author details

Mónica Roxo-Rosa[1], Mónica Oleastro[2] and Ana Isabel Lopes[3]

*Address all correspondence to: roxorosa@hotmail.com

1 Center for Biodiversity, Functional & Integrative Genomics, Faculty of Sciences, University of Lisbon, Lisbon, Portugal and Department of Genetics, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal

2 Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal

3 Gastroenterology Unit, Department of Paediatrics, University Hospital Santa Maria, Medical Faculty of Lisbon, Lisbon, Portugal

References

[1] Linz B, Balloux F, Moodley Y, Manica A, Liu H, Roumagnac P et al. An African origin for the intimate association between humans and Helicobacter pylori. Nature 2007; 445(7130):915-918.

[2] Blaser MJ. Helicobacters are indigenous to the human stomach: duodenal ulceration is due to changes in gastric microecology in the modern era. Gut 1998; 43(5):721-727.

[3] Konturek PC, Konturek SJ, Brzozowski T. Helicobacter pylori infection in gastric cancerogenesis. J Physiol Pharmacol 2009; 60(3):3-21.

[4] Pacifico L, Anania C, Osborn JF, Ferraro F, Chiesa C. Consequences of Helicobacter pylori infection in children. World J Gastroenterol 2010; 16(41):5181-5194.

[5] Oderda G, Shcherbakov P, Bontems P, Urruzuno P, Romano C, Gottrand F et al. Results from the pediatric European register for treatment of Helicobacter pylori (PERTH). Helicobacter 2007; 12(2):150-156.

[6] Oderda G, Mura S, Valori A, Brustia R. Idiopathic peptic ulcers in children. J Pediatr Gastroenterol Nutr 2009; 48(3):268-270.

[7] Tam YH, Lee KH, To KF, Chan KW, Cheung ST. Helicobacter pylori-positive versus Helicobacter pylori-negative idiopathic peptic ulcers in children with their long-term outcomes. J Pediatr Gastroenterol Nutr 2009; 48(3):299-305.

[8] Kalach N, Bontems P, Koletzko S, Mourad-Baars P, Shcherbakov P, Celinska-Cedro D et al. Frequency and risk factors of gastric and duodenal ulcers or erosions in children: a prospective 1-month European multicenter study. Eur J Gastroenterol Hepatol 2010; 22(10):1174-1181.

[9] Oleastro M, Cordeiro R, Ferrand J, Nunes B, Lehours P, Carvalho-Oliveira I et al. Evaluation of the clinical significance of homB, a novel candidate marker of Helicobacter pylori strains associated with peptic ulcer disease. J Infect Dis 2008; 198(9): 1379-1387.

[10] Lopes AI, Palha A, Monteiro L, Olcastro M, Pelerito A, Fernandes A. Helicobacter pylori genotypes in children from a population at high gastric cancer risk: no association with gastroduodenal histopathology. Am J Gastroenterol 2006; 101(9):2113-2122.

[11] Oleastro M, Monteiro L, Lehours P, Megraud F, Menard A. Identification of markers for Helicobacter pylori strains isolated from children with peptic ulcer disease by suppressive subtractive hybridization. Infect Immun 2006; 74(7):4064-4074.

[12] Oleastro M, Cordeiro R, Yamaoka Y, Queiroz D, Megraud F, Monteiro L et al. Disease association with two Helicobacter pylori duplicate outer membrane protein genes, homB and homA. Gut Pathog 2009; 1(1):12.

[13] Oleastro M, Santos A, Cordeiro R, Nunes B, Megraud F, Menard A. Clinical relevance and diversity of two homologous genes encoding glycosyltransferases in Helicobacter pylori. J Clin Microbiol 2010; 48(8):2885-2891.

[14] Oleastro M, Gerhard M, Lopes AI, Ramalho P, Cabral J, Sousa GA et al. Helicobacter pylori virulence genotypes in Portuguese children and adults with gastroduodenal pathology. Eur J Clin Microbiol Infect Dis 2003; 22(2):85-91.

[15] Oleastro M, Cordeiro R, Menard A, Yamaoka Y, Queiroz D, Megraud F et al. Allelic diversity and phylogeny of homB, a novel co-virulence marker of Helicobacter pylori. BMC Microbiol 2009; 9(1):248.

[16] Vitoriano I, Saraiva-Pava KD, Rocha-Goncalves A, Santos A, Lopes AI, Oleastro M et al. Ulcerogenic Helicobacter pylori strains isolated from children: a contribution to get insight into the virulence of the bacteria. PLoS ONE 2011; 6(10):e26265.

[17] Linden S, Semino-Mora C, Liu H, Rick J, Dubois A. Role of mucin Lewis status in resistance to Helicobacter pylori infection in pediatric patients. Helicobacter 2010; 15(4):251-258.

[18] Lopes AI, Quiding-Jarbrink M, Palha A, Ruivo J, Monteiro L, Oleastro M et al. Cytokine expression in pediatric Helicobacter pylori infection. Clin Diagn Lab Immunol 2005; 12(8):994-1002.

[19] Lopes AI, Victorino RM, Palha AM, Ruivo J, Fernandes A. Mucosal lymphocyte subsets and HLA-DR antigen expression in paediatric Helicobacter pylori-associated gastritis. Clin Exp Immunol 2006; 145(1):13-20.

[20] Salles N, Megraud F. Current management of Helicobacter pylori infections in the elderly. Expert Rev Anti Infect Ther 2007; 5(5):845-856.

[21] Rowland M, Daly L, Vaughan M, Higgins A, Bourke B, Drumm B. Age-specific incidence of Helicobacter pylori. Gastroenterology 2006; 130(1):65-72.

[22] Goodman KJ, O'rourke K, Day RS, Wang C, Nurgalieva Z, Phillips CV et al. Dynamics of Helicobacter pylori infection in a US-Mexico cohort during the first two years of life. Int J Epidemiol 2005; 34(6):1348-1355.

[23] Mitchell H, Megraud F. Epidemiology and diagnosis of Helicobacter pylori infection. Helicobacter 2002; 7 Suppl 1:8-16.

[24] Bardhan PK. Epidemiological features of Helicobacter pylori infection in developing countries. Clin Infect Dis 1997; 25(5):973-978.

[25] Kawakami E, Machado RS, Ogata SK, Langner M. Decrease in prevalence of Helicobacter pylori infection during a 10-year period in Brazilian children. Arq Gastroenterol 2008; 45(2):147-151.

[26] Elitsur Y, Dementieva Y, Rewalt M, Lawrence Z. Helicobacter pylori infection rate decreases in symptomatic children: a retrospective analysis of 13 years (1993-2005) from a gastroenterology clinic in West Virginia. J Clin Gastroenterol 2009; 43(2): 147-151.

[27] Azevedo NF, Huntington J, Goodman KJ. The epidemiology of Helicobacter pylori and public health implications. Helicobacter 2009; 14 Suppl 1:1-7.

[28] Oleastro M, Pelerito A, Nogueira P, Benoliel J, Santos A, Cabral J et al. Prevalence and incidence of Helicobacter pylori Infection in a healthy pediatric population in the Lisbon area. Helicobacter 2011; 16(5):363-372.

[29] Quina MG. Helicobacter pylori: the Portuguese scene. Grupo de Estudo Portugues do Helicobacter pylori (GEPHP). Eur J Cancer Prev 1994; 3 Suppl 2:65-67.

[30] Vitoriano I, Rocha-Goncalves A, Carvalho T, Oleastro M, Calado CR, Roxo-Rosa M. Antigenic Diversity Among Portuguese Clinical Isolates of Helicobacter pylori. Helicobacter 2011; 16(2):153-168.

[31] Lopes AI, Oleastro M, Palha A, Fernandes A, Monteiro L. Antibiotic-resistant Helicobacter pylori strains in Portuguese children. Pediatr Infect Dis J 2005; 24(5):404-409.

[32] Oleastro M, Cabral J, Ramalho PM, Lemos PS, Paixao E, Benoliel J et al. Primary antibiotic resistance of Helicobacter pylori strains isolated from Portuguese children: a prospective multicentre study over a 10 year period. J Antimicrob Chemother 2011; 66(10):2308-2311.

[33] Vale FF, Roxo-Rosa M, Oleastro M. *Helicobacter pylori* resistance to antibiotics. In: A.Méndez-Vilas. (ed.) Science against microbial pathogens: communicating current research and technological advances. 2011. p745-756.

[34] Megraud F, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM et al. Helicobacter pylori resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut 2013; 62(1):34-42.

[35] De F, V, Giorgio F, Hassan C, Manes G, Vannella L, Panella C et al. Worldwide H. pylori antibiotic resistance: a systematic review. J Gastrointestin Liver Dis 2010; 19(4): 409-414.

[36] Yamade M, Sugimoto M, Uotani T, Nishino M, Kodaira C, Furuta T. Resistance of Helicobacter pylori to quinolones and clarithromycin assessed by genetic testing in Japan. J Gastroenterol Hepatol 2011; 26(9):1457-1461.

[37] Koletzko S, Richy F, Bontems P, Crone J, Kalach N, Monteiro ML et al. Prospective multicentre study on antibiotic resistance of Helicobacter pylori strains obtained from children living in Europe. Gut 2006; 55(12):1711-1716.

[38] Agudo S, Alarcon T, Cibrelus L, Urruzuno P, Martinez MJ, Lopez-Brea M. [High percentage of clarithromycin and metronidazole resistance in Helicobacter pylori clinical isolates obtained from Spanish children]. Rev Esp Quimioter 2009; 22(2):88-92.

[39] Stingl K, De Reuse H. Staying alive overdosed: how does Helicobacter pylori control urease activity? Int J Med Microbiol 2005; 295(5):307-315.

[40] Benoit SL, Maier RJ. Mua (HP0868) is a nickel-binding protein that modulates urease activity in Helicobacter pylori. MBio 2011; 2(2):e00039-11.

[41] Schreiber S, Konradt M, Groll C, Scheid P, Hanauer G, Werling HO et al. The spatial orientation of Helicobacter pylori in the gastric mucus. Proc Natl Acad Sci U S A 2004; 101(14):5024-5029.

[42] Ottemann KM, Lowenthal AC. Helicobacter pylori uses motility for initial colonization and to attain robust infection. Infect Immun 2002; 70(4):1984-1990.

[43] Hazell SL, Lee A, Brady L, Hennessy W. Campylobacter pyloridis and gastritis: association with intercellular spaces and adaptation to an environment of mucus as im-

portant factors in colonization of the gastric epithelium. J Infect Dis 1986; 153(4): 658-663.

[44] Sycuro LK, Pincus Z, Gutierrez KD, Biboy J, Stern CA, Vollmer W et al. Peptidoglycan crosslinking relaxation promotes Helicobacter pylori's helical shape and stomach colonization. Cell 2010; 141(5):822-833.

[45] Josenhans C, Labigne A, Suerbaum S. Comparative ultrastructural and functional studies of Helicobacter pylori and Helicobacter mustelae flagellin mutants: both flagellin subunits, FlaA and FlaB, are necessary for full motility in Helicobacter species. J Bacteriol 1995; 177(11):3010-3020.

[46] Eaton KA, Suerbaum S, Josenhans C, Krakowka S. Colonization of gnotobiotic piglets by Helicobacter pylori deficient in two flagellin genes. Infect Immun 1996; 64(7): 2445-2448.

[47] Watanabe S, Takagi A, Tada U, Kabir AM, Koga Y, Kamiya S et al. Cytotoxicity and motility of Helicobacter pylori. PG - S169-71 AB - To clarify the relationship between interleukin-8 (IL-8) production and virulent factors, we examined the motility and cytotoxicity of H. pylori, suggested to be a major cause of chronic gastritis and peptic ulcers. Our results demonstrated that among cytotoxic strains of H. pylori, high-motility strains induced more IL-8 than low-motility strains. There was no correlation between cytotoxicity and motility of H. pylori. Four restriction fragment length polymorphism (RFLP) patterns were observed in the flaA PCR products. There was no correlation between flaA RFLP and motility. In conclusion, our findings suggest that both cytotoxicity and motility are virulent factors in the pathogenesis of gastric mucosal injury. J Clin Gastroenterol 1997; 25 Suppl 1.

[48] Dubois A, Boren T. Helicobacter pylori is invasive and it may be a facultative intracellular organism. Cell Microbiol 2007; 9(5):1108-1116.

[49] Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET et al. Helicobacter pylori adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science 1998; 279(5349):373-377.

[50] Ohno T, Vallstrom A, Rugge M, Ota H, Graham DY, Arnqvist A et al. Effects of blood group antigen-binding adhesin expression during Helicobacter pylori infection of Mongolian gerbils. J Infect Dis 2011; 203(5):726-735.

[51] Fujimoto S, Olaniyi OO, Arnqvist A, Wu JY, Odenbreit S, Haas R et al. Helicobacter pylori BabA expression, gastric mucosal injury, and clinical outcome. Clin Gastroenterol Hepatol 2007; 5(1):49-58.

[52] Mahdavi J, Sonden B, Hurtig M, Olfat FO, Forsberg L, Roche N et al. Helicobacter pylori SabA adhesin in persistent infection and chronic inflammation. Science 2002; 297(5581):573-578.

[53] Sakamoto S, Watanabe T, Tokumaru T, Takagi H, Nakazato H, Lloyd KO. Expression of Lewisa, Lewisb, Lewisx, Lewisy, siayl-Lewisa, and sialyl-Lewisx blood group anti-

gens in human gastric carcinoma and in normal gastric tissue. Cancer Res 1989; 49(3): 745-752.

[54] Ota H, Nakayama J, Momose M, Hayama M, Akamatsu T, Katsuyama T et al. Helicobacter pylori infection produces reversible glycosylation changes to gastric mucins. Virchows Arch 1998; 433(5):419-426.

[55] Aspholm M, Olfat FO, Norden J, Sonden B, Lundberg C, Sjostrom R et al. SabA is the H. pylori hemagglutinin and is polymorphic in binding to sialylated glycans. PLoS Pathog 2006; 2(10):e110.

[56] Unemo M, Aspholm-Hurtig M, Ilver D, Bergstrom J, Boren T, Danielsson D et al. The sialic acid binding SabA adhesin of Helicobacter pylori is essential for nonopsonic activation of human neutrophils. J Biol Chem 2005; 280(15):15390-15397.

[57] Yamaoka Y, Kwon DH, Graham DY. A M(r) 34,000 proinflammatory outer membrane protein (oipA) of Helicobacter pylori. Proc Natl Acad Sci U S A 2000; 97(13): 7533-7538.

[58] Yamaoka Y, Ojo O, Fujimoto S, Odenbreit S, Haas R, Gutierrez O et al. Helicobacter pylori outer membrane proteins and gastroduodenal disease. Gut 2006; 55(6):775-781.

[59] Franco AT, Johnston E, Krishna U, Yamaoka Y, Israel DA, Nagy TA et al. Regulation of gastric carcinogenesis by Helicobacter pylori virulence factors. Cancer Res 2008; 68(2):379-387.

[60] Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M et al. cag, a pathogenicity island of Helicobacter pylori, encodes type I-specific and disease-associated virulence factors. Proc Natl Acad Sci U S A 1996; 93(25):14648-14653.

[61] Covacci A, Rappuoli R. Tyrosine-phosphorylated bacterial proteins: Trojan horses for the host cell. J Exp Med 2000; 191(4):587-592.

[62] Parker H, Keenan JI. Composition and function of Helicobacter pylori outer membrane vesicles. Microbes Infect 2012; 14(1):9-16.

[63] Backert S, Tegtmeyer N, Selbach M. The versatility of Helicobacter pylori CagA effector protein functions: The master key hypothesis. Helicobacter 2010; 15(3):163-176.

[64] Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH et al. Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res 1995; 55(10): 2111-2115.

[65] Covacci A, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G et al. Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer. Proc Natl Acad Sci U S A 1993; 90(12): 5791-5795.

[66] Ohnishi N, Yuasa H, Tanaka S, Sawa H, Miura M, Matsui A et al. Transgenic expression of Helicobacter pylori CagA induces gastrointestinal and hematopoietic neoplasms in mouse. Proc Natl Acad Sci U S A 2008; 105(3):1003-1008.

[67] Ferreira RM, Machado JC, Figueiredo C. Variation in Helicobacter pylori cagA promoter region is associated with CagA expression. Helicobacter 2012; 17(S1):W1.5.

[68] Palframan SL, Kwok T, Gabriel K. Vacuolating cytotoxin A (VacA), a key toxin for Helicobacter pylori pathogenesis. Front Cell Infect Microbiol 2012; 2:92.

[69] Chambers MG, Pyburn TM, Gonzalez-Rivera C, Collier SE, Eli I, Yip CK et al. Structural Analysis of the Oligomeric States of Helicobacter pylori VacA Toxin. J Mol Biol 2012.

[70] Gangwer KA, Mushrush DJ, Stauff DL, Spiller B, McClain MS, Cover TL et al. Crystal structure of the Helicobacter pylori vacuolating toxin p55 domain. Proc Natl Acad Sci U S A 2007; 104(41):16293-16298.

[71] Boquet P, Ricci V, Galmiche A, Gauthier NC. Gastric cell apoptosis and H. pylori: has the main function of VacA finally been identified? Trends Microbiol 2003; 11(9): 410-413.

[72] Cover TL, Blanke SR. Helicobacter pylori VacA, a paradigm for toxin multifunctionality. Nat Rev Microbiol 2005; 3(4):320-332.

[73] Rieder G, Fischer W, Haas R. Interaction of Helicobacter pylori with host cells: function of secreted and translocated molecules. Curr Opin Microbiol 2005; 8(1):67-73.

[74] Yamaoka Y. Pathogenesis of Helicobacter pylori-Related Gastroduodenal Diseases from Molecular Epidemiological Studies. Gastroenterol Res Pract 2012; 2012:371503.

[75] Tham KT, Peek RM, Jr., Atherton JC, Cover TL, Perez-Perez GI, Shyr Y et al. Helicobacter pylori genotypes, host factors, and gastric mucosal histopathology in peptic ulcer disease. Hum Pathol 2001; 32(3):264-273.

[76] Israel DA, Peek RM. Surreptitious manipulation of the human host by Helicobacter pylori. Gut Microbes 2010; 1(2):119-127.

[77] Moran AP, Hynes SO, Heneghan MA. Mimicry of blood group antigen A by Helicobacter mustelae and H. pylori. Gastroenterology 1999; 116(2):504-505.

[78] Jonsson K, Guo BP, Monstein HJ, Mekalanos JJ, Kronvall G. Molecular cloning and characterization of two Helicobacter pylori genes coding for plasminogen-binding proteins. Proc Natl Acad Sci U S A 2004; 101(7):1852-1857.

[79] Axsen WS, Styer CM, Solnick JV. Inhibition of heat shock protein expression by Helicobacter pylori. Microb Pathog 2009; 47(4):231-236.

[80] Solnick JV, Hansen LM, Salama NR, Boonjakuakul JK, Syvanen M. Modification of Helicobacter pylori outer membrane protein expression during experimental infection of rhesus macaques. Proc Natl Acad Sci U S A 2004; 101(7):2106-2111.

[81] Carvalho MA, Machado NC, Ortolan EV, Rodrigues MA. Upper gastrointestinal histopathological findings in children and adolescents with nonulcer dyspepsia with Helicobacter pylori infection. J Pediatr Gastroenterol Nutr 2012; 55(5):523-529.

[82] Sood MR, Joshi S, Akobeng AK, Mitchell J, Thomas AG. Growth in children with Helicobacter pylori infection and dyspepsia. Arch Dis Child 2005; 90(10):1025-1028.

[83] Yang YJ, Sheu BS, Lee SC, Yang HB, Wu JJ. Children of Helicobacter pylori-infected dyspeptic mothers are predisposed to H. pylori acquisition with subsequent iron deficiency and growth retardation. Helicobacter 2005; 10(3):249-255.

[84] Farrell S, Milliken I, Murphy JL, Wootton SA, McCallion WA. Nonulcer dyspepsia and Helicobacter pylori eradication in children. J Pediatr Surg 2005; 40(10):1547-1550.

[85] Kalach N, Mention K, Guimber D, Michaud L, Spyckerelle C, Gottrand F. Helicobacter pylori infection is not associated with specific symptoms in nonulcer-dyspeptic children. Pediatrics 2005; 115(1):17-21.

[86] Bourke B, Ceponis P, Chiba N, Czinn S, Ferraro R, Fischbach L et al. Canadian Helicobacter Study Group Consensus Conference: Update on the approach to Helicobacter pylori infection in children and adolescents--an evidence-based evaluation. Can J Gastroenterol 2005; 19(7):399-408.

[87] Malfertheiner P, Megraud F, O'Morain C, Bazzoli F, El Omar E, Graham D et al. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. Gut 2007; 56(6):772-781.

[88] Tindberg Y, Nyren O, Blennow M, Granstrom M. Helicobacter pylori infection and abdominal symptoms among Swedish school children. J Pediatr Gastroenterol Nutr 2005; 41(1):33-38.

[89] Bode G, Brenner H, Adler G, Rothenbacher D. Recurrent abdominal pain in children: evidence from a population-based study that social and familial factors play a major role but not Helicobacter pylori infection. J Psychosom Res 2003; 54(5):417-421.

[90] Perez ME, Youssef NN. Dyspepsia in childhood and adolescence: insights and treatment considerations. Curr Gastroenterol Rep 2007; 9(6):447-455.

[91] Koletzko S, Jones NL, Goodman KJ, Gold B, Rowland M, Cadranel S et al. Evidence-based guidelines from ESPGHAN and NASPGHAN for Helicobacter pylori infection in children. J Pediatr Gastroenterol Nutr 2011; 53(2):230-243.

[92] Drumm B, Rhoads JM, Stringer DA, Sherman PM, Ellis LE, Durie PR. Peptic ulcer disease in children: etiology, clinical findings, and clinical course. Pediatrics 1988; 82(3 Pt 2):410-414.

[93] Ubukata H, Nagata H, Tabuchi T, Konishi S, Kasuga T, Tabuchi T. Why is the coexistence of gastric cancer and duodenal ulcer rare? Examination of factors related to both gastric cancer and duodenal ulcer. Gastric Cancer 2011; 14(1):4-12.

[94] Arents NL, Thijs JC, van Zwet AA, Kleibeuker JH. Does the declining prevalence of Helicobacter pylori unmask patients with idiopathic peptic ulcer disease? Trends over an 8 year period. Eur J Gastroenterol Hepatol 2004; 16(8):779-783.

[95] Vakil N. Dyspepsia, peptic ulcer, and H. pylori: a remembrance of things past. Am J Gastroenterol 2010; 105(3):572-574.

[96] Sherman P, Czinn S, Drumm B, Gottrand F, Kawakami E, Madrazo A et al. Helicobacter pylori infection in children and adolescents: Working Group Report of the First World Congress of Pediatric Gastroenterology, Hepatology, and Nutrition. J Pediatr Gastroenterol Nutr 2002; 35 Suppl 2:S128-S133.

[97] Bourke B, Sherman P, Drumm B. Peptic ulcer disease: what is the role for Helicobacter pylori? Semin Gastrointest Dis 1994; 5(1):24-31.

[98] Kneepkens C.M.F. Peptic ulcer disease in childhood. Acta Endosc 1994; 24:169-181.

[99] Oderda G, Ansaldi N. Peptic ulcers in childhood. Lancet 1988; 1(8580):302-303.

[100] Egbaria R, Levine A, Tamir A, Shaoul R. Peptic ulcers and erosions are common in Israeli children undergoing upper endoscopy. Helicobacter 2008; 13(1):62-68.

[101] Kato S, Nishino Y, Ozawa K, Konno M, Maisawa S, Toyoda S et al. The prevalence of Helicobacter pylori in Japanese children with gastritis or peptic ulcer disease. J Gastroenterol 2004; 39(8):734-738.

[102] Nijevitch AA, Sataev VU, Vakhitov VA, Loguinovskaya VV, Kotsenko TM. Childhood peptic ulcer in the Ural area of Russia: clinical status and Helicobacter pylori-associated immune response. J Pediatr Gastroenterol Nutr 2001; 33(5):558-564.

[103] Walsh JH, Peterson WL. The treatment of Helicobacter pylori infection in the management of peptic ulcer disease. N Engl J Med 1995; 333(15):984-991.

[104] Macarthur C, Saunders N, Feldman W. Helicobacter pylori, gastroduodenal disease, and recurrent abdominal pain in children. JAMA 1995; 273(9):729-734.

[105] Brown K, Lundborg P, Levinson J, Yang H. Incidence of peptic ulcer bleeding in the US pediatric population. J Pediatr Gastroenterol Nutr 2012; 54(6):733-736.

[106] Khurana R, Fischbach L, Chiba N, VAN Zanten SV, Sherman PM, George BA et al. Meta-analysis: Helicobacter pylori eradication treatment efficacy in children. Aliment Pharmacol Ther 2007; 25(5):523-536.

[107] Ford AC, Delaney BC, Forman D, Moayyedi P. Eradication therapy for peptic ulcer disease in Helicobacter pylori positive patients. Cochrane Database Syst Rev 2006; (2):CD003840.

[108] Kato S, Sherman PM. What is new related to Helicobacter pylori infection in children and teenagers? Arch Pediatr Adolesc Med 2005; 159(5):415-421.

[109] Goggin N, Rowland M, Imrie C, Walsh D, Clyne M, Drumm B. Effect of Helicobacter pylori eradication on the natural history of duodenal ulcer disease. Arch Dis Child 1998; 79(6):502-505.

[110] Chiesa C, Pacifico L, Anania C, Poggiogalle E, Chiarelli F, Osborn JF. Helicobacter pylori therapy in children: overview and challenges. Int J Immunopathol Pharmacol 2010; 23(2):405-416.

[111] Sherman P, Hassall E, Hunt RH, Fallone CA, Veldhuyzen VZ, Thomson AB. Canadian Helicobacter Study Group Consensus Conference on the Approach to Helicobacter pylori Infection in Children and Adolescents. Can J Gastroenterol 1999; 13(7): 553-559.

[112] Huang FC, Chang MH, Hsu HY, Lee PI, Shun CT. Long-term follow-up of duodenal ulcer in children before and after eradication of Helicobacter pylori. J Pediatr Gastroenterol Nutr 1999; 28(1):76-80.

[113] Tytgat GN. Etiopathogenetic principles and peptic ulcer disease classification. Dig Dis 2011; 29(5):454-458.

[114] Atherton JC, Blaser MJ. Coadaptation of Helicobacter pylori and humans: ancient history, modern implications. J Clin Invest 2009; 119(9):2475-2487.

[115] Osefo N, Ito T, Jensen RT. Gastric acid hypersecretory states: recent insights and advances. Curr Gastroenterol Rep 2009; 11(6):433-441.

[116] El Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 2000; 404(6776):398-402.

[117] Hansson LE, Nyren O, Hsing AW, Bergstrom R, Josefsson S, Chow WH et al. The risk of stomach cancer in patients with gastric or duodenal ulcer disease. N Engl J Med 1996; 335(4):242-249.

[118] Whitney AE, Guarner J, Hutwagner L, Gold BD. Helicobacter pylori gastritis in children and adults: comparative histopathologic study. Ann Diagn Pathol 2000; 4(5): 279-285.

[119] de Oliveira AM, Rocha GA, Queiroz DM, Mendes EN, de Carvalho AS, Ferrari TC et al. Evaluation of enzyme-linked immunosorbent assay for the diagnosis of Helicobacter pylori infection in children from different age groups with and without duodenal ulcer. J Pediatr Gastroenterol Nutr 1999; 28(2):157-161.

[120] Oderda G, Vaira D, Holton J, Ainley C, Altare F, Boero M et al. Helicobacter pylori in children with peptic ulcer and their families. Dig Dis Sci 1991; 36(5):572-576.

[121] Lee A. Future research in peptic ulcer disease. Scand J Gastroenterol Suppl 1994; 205:51-58.

[122] Drumm B, Day AS, Gold B, Gottrand F, Kato S, Kawakami E et al. Helicobacter pylori and peptic ulcer: Working Group Report of the second World Congress of Pediatric Gastroenterology, Hepatology, and Nutrition. J Pediatr Gastroenterol Nutr 2004; 39 Suppl 2:S626-S631.

[123] Schubert TT, Bologna SD, Nensey Y, Schubert AB, Mascha EJ, Ma CK. Ulcer risk factors: interactions between Helicobacter pylori infection, nonsteroidal use, and age. Am J Med 1993; 94(4):413-418.

[124] Niv Y, Boltin D. Secreted and membrane-bound mucins and idiopathic peptic ulcer disease. Digestion 2012; 86(3):258-263.

[125] Yamashiro Y, Oguchi S, Otsuka Y, Nagata S, Shioya T, Shimizu T. Helicobacter pylori colonization in children with gastritis and peptic ulcer. I. The colonization rate and effects of colonization on mucin content and mucosal inflammation in the antrum. Acta Paediatr Jpn 1994; 36(2):167-170.

[126] Rad R, Prinz C, Neu B, Neuhofer M, Zeitner M, Voland P et al. Synergistic effect of Helicobacter pylori virulence factors and interleukin-1 polymorphisms for the development of severe histological changes in the gastric mucosa. J Infect Dis 2003; 188(2): 272-281.

[127] Wilschanski M, Schlesinger Y, Faber J, Rudensky B, Ohnona FS, Freier S et al. Combination of Helicobacter pylori strain and tumor necrosis factor-alpha polymorphism of the host increases the risk of peptic ulcer disease in children. J Pediatr Gastroenterol Nutr 2007; 45(2):199-203.

[128] Moura SB, Almeida LR, Guerra JB, Rocha GA, Camargos Rocha AM, Melo FF et al. Toll-like receptor (TLR2, TLR4 and TLR5) gene polymorphisms and Helicobacter pylori infection in children with and without duodenal ulcer. Microbes Infect 2008; 10(14-15):1477-1483.

[129] Jungblut PR, Pleissner KP, Stein R, Butnner S, Knipper J. http://web.mpiib-berlin.mpg.de/cgi-bin/pdbs/2d-page/extern/index.cgi. Proteome 2D-PAGE Database - Home

[130] Kao CY, Sheu BS, Sheu SM, Yang HB, Chang WL, Cheng HC et al. Higher motility enhances bacterial density and inflammatory response in dyspeptic patients infected with Helicobacter pylori. Helicobacter 2012; 17(6):411-416.

IntechOpen

IntechOpen