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Significance of cagA Status and vacA Subtypes of Helicobacter pylori in Determining Gastric Histopathology in Pakistan

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histopathology, culture, and real-time polymerase chain reaction (PCR), whereas the serum samples were used in enzyme-linked immunosorbent assay (ELISA).
Results. Real-time PCR had the highest sensitivity (100%), followed by culture (86.9%), histopathology (83.7%), rapid urease test (82.6%), and ELISA (76.0%). Of the biopsy specimens tested independent of pathology, 93.5% were *cagA* positive. The *vacA* s1-m2 genotype was the predominant subtype, found in 63.3% of the patients with CG and 68.7% of those with DU. Most *H. pylori* strains in CG (66.6%) were *iceA2* positive, whereas *iceA1* was predominant in DU (68.7%). In terms of the association between *iceA* alleles with other genes in this study, both alleles were significantly associated with the *cagA*-positive and *vacA* s1-m2 genotype.
Conclusion. All genotypes can be directly detected in *H. pylori* strains isolated from single gastric biopsy specimen by real-time PCR. The prevalent circulating genotypes in CG and DU were *cagA vacA* s1m2 *iceA2*, and *cagA vacA* s1m2 *iceA1* genotype, respectively. It was found that *cagA vacA* s1m2 genotype seems to be common virulence factors in both CG and DU, whereas *iceA* alleles show specificity for gastroduodenal pathologies in this study.

Abstract no.: P019
Cloning and Expression of UreB-Derived Fragment of *Helicobacter pylori* Urease

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The Urease activity of *H. pylori* plays an important role in its pathogenicity and is essential for the survival and colonization of the stomach. The urease is composed of A and B subunit, of which the urease subunit B (UreB) is considered as an excellent vaccine candidate antigen and found to be protective in mice but because of immunosuppressant effect of whole UreB, we select a fragment of this protein and cloned and expressed it.
Material and Method. We select a domain of 122 amino acid of UreB protein and cloned it in pET-32a vector and expressed it in BL21-(DE3) and confirm it with anti His-tag antibody.
Discussion. Urease has the ability to bind class II MHC and induce apoptosis in class II MHC-bearing cells so complete urease has restricted as a vaccine candidate. The development of Abs that would block the binding of urease to class II MHC may effectively prevent bacterial binding to the target tissue and colonization, and reduce the cellular injury. Thus, efforts are currently directed at mapping the sites of urease contact with human class II MHC molecules. So we produced a recombinant UreB that can be used as a candidate for more immunologic and protective effect studies.

Poster Round 1.3: Virulence Factors and Pathogenesis

Abstract no.: P020
Helicobacter pylori Delocalizes Tight Junction Molecules

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The clinical outcomes of the infection by the pathogen *Helicobacter pylori* result from the interaction between the bacteria and the gastric epithelial cells. The process is not completely understood but there are some studies indicating that the loss of the gastric epithelial barrier caused by the bacteria may play a role in it. Tight junctions are the intercellular structures responsible for the barrier function of the epithelium.

In the present study, we have examined the localization of the tight junction molecules occludin, claudin-4, and ZO-1 in Caco-2, T84, and NCI-N87 cell lines with and without *H. pylori*. We found that *H. pylori* strains 26695 and 60190 were able to delocalize occludin, claudin-4, and ZO-1 to the cytoplasm with the concomitant weakening of the staining in the membrane and the appearance of a punctuate pattern in the cytoplasm. This activity of *H. pylori* appeared to be independent of the well-characterized virulence factors VacA and CagA. In conclusion, we saw that *H. pylori* disturbs the organization of the tight junctions of epithelial cells in vitro. FCT POCI/SAU-IMI/56681/2004; FCG 356327-S.

Abstract no.: P021
The Effect of *Helicobacter pylori* Infection on Sonic Hedgehog Signal Pathways in Gastric Epithelial Cells

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Background/Aims. Aberrant activation of hedgehog signaling leads to pathological consequences in human tumors. Sonic hedgehog (Shh) is implicated in stem/progenitor cell restitution of damaged gastric mucosa during chronic infection. *H. pylori* causes mucosal damage and may participate in gastric carcinogenesis.

The aim of study was to examine the changes in Shh signal pathways in response to *H. pylori* infection.
Methods. Real-time polymerase chain reaction (RT-PCR), Western blot, and immunohistochemistry were utilized to analyze the expression, localization, and transcriptional regulation of Shh, Ptch, and Gli-1. 60190, 8822, And gene knock-out mutant strain of *cagA* were used. AGS, MKN74, and MKN45 cells were used. Paraffin-embedded tissues from resected human gastric cancer were used to evaluate the expression of Shh and related signal proteins in vivo.
Results. Expression of Shh in gastric epithelial cells infected with *H. pylori* was increased confirmed by immunoblot and RT-PCR. Furthermore, *cagA*-positive strains showed higher level of Shh. Gli-1, the transcriptional target of Hh pathway, was increased in cells infected with *H. pylori* compare to uninfected control implying that *H. pylori* might lead to enhanced Hh pathway in gastric cancer. Shh-positive immunoexpression in *H. pylori*-infected gastric cancer tissue was higher than uninfected tissue. Immunostaining of Shh correlated with tumor differentiation apart from the status of *H. pylori* infection.
Conclusion. Shh expression correlates with the status of *H. pylori* infection, and *H. pylori* may activate the Hh pathways through the up-regulation of Shh in gastric epithelial cells. CagA may influence the expression of Shh in gastric epithelial cells.

Abstract no.: P022
Disruption of Host Cell Signaling Pathways Induced by *Helicobacter pylori* Resulting in Hummingbird Phenotype

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Cell lines infected with *Helicobacter pylori* have shown that the translocation of cytotoxin-associated gene A antigen (CagA) alone into host cells can cause them to become elongated, resembling a hummingbird beak appearance. It has been reported that this "hummingbird" phenotype correlates with pathogenesis but the host's signaling mechanisms exploited by *H. pylori* to result in the "hummingbird" phenotype are poorly understood. This study sets out to investigate the effect of CagA expression and the contribution of two major intracellular signaling pathways, namely the MAP kinases and phosphoinositide 3-kinase (PI3K) in causing the "hummingbird" phenotype. Results from the study show that both MAP kinases (ERK, p38, and JNK) and PI3K pathways were activated in *H. pylori*-infected and CagA-transfected AGS cells. Interestingly, using specific chemical inhibitors, ERK activity but not that of p38, JNK, and PI3K, was found to be required for the induction of the "hummingbird" phenotype. In conclusion, this study indicates CagA modulates intracellular signaling, in particularly the ERK MAP kinase pathway of host cells to mediate *H. pylori* pathogenesis.

Abstract no.: P023
Are CagA-positive Strains of *Helicobacter pylori* Associated with Increased Risk of Ischemic Heart Disease? A Meta-Analysis

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Previous studies and meta-analyses on the association between ischemic heart disease (IHD) and to *H. pylori* reported conflicting results. Conversely, some pilot studies showed that the association between IHD and *H. pylori* is stronger after stratifying the data for the prevalence of CagA-positive strains. Nevertheless, this association remains still debated. Therefore, we designed a study aimed at analyzing the effect of CagA-positive strains on acute coronary syndromes. We performed a meta-analysis including 13 epidemiological studies (3 prospective and 10 retrospective), involving a total of 5503 patients on the association between seropositivity to CagA and IHD. We only selected studies including patients admitted for an acute coronary syndrome, whereas studies including patients with stable angina or other chronic coronary disease were excluded. Seropositivity to CagA was significantly associated with the occurrence of acute coronary syndromes: 1.49 (1.21–1.82) (random OR, 95% CI), $p = .0001$. Heterogeneity was found, $\chi^2 = 19.5$, $p = .08$. A sensitivity analysis excluding studies that gave the individual strongest positive association and with a large CI and a small sample size confirmed the main result. A prespecified subgroup analysis according to the type of study, whether retrospective or prospective, confirmed the global meta-analysis results in both subgroups. The association remained significant even after the adjustment for potential confounding factors. This meta-analysis shows the presence of a significant association between seropositivity to CagA and the occurrence of acute coronary syndromes. Further studies are needed to better elucidate the mechanisms by which CagA-positive strains may affect coronary atherosclerotic disease.

Abstract no.: P024
Cell-Surface Mannan Polysaccharide in *Helicobacter pylori*

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Polysaccharides are one of the main components of bacterial cell walls. With the intent of formulating a multivalent *Helicobacter pylori* lipopolysaccharide-based vaccine, several studies have been carried by Monteiro and coworkers in order to understand the structural variability of *H. pylori* cell-surface glycans. It is now well documented

that *H. pylori* produces cell-surface lipopolysaccharides (LPSs) composed of structures homologous to mammalian blood group antigens [1], termed "Lewis O-chains," that are thought to play a role in *H. pylori* pathogenesis. These studies have also demonstrated that wild-type *H. pylori* LPS isolates contain small amounts of mannose, but, so far, a mannose-rich polysaccharide was only detected in a LPS mutant strain [2].

In this work we observed that wild-type *H. pylori* strain 968 produces a mannose-rich polysaccharide coexpressed with a "Lewis O-chain" polysaccharide. Fine structural studies comprising chemical and spectroscopy methods demonstrated that this mannan is composed of a linear backbone of α-(1→2)-linked mannose units in which approximately 50% is substituted at the *O*-6 position. This mannan may represent an *H. pylori*-specific exopolysaccharide that may be expressed by many strains.

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Abstract no.: P025
Adherence of *Helicobacter pylori* on Murine Mesenchymal Stem Cells

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Background. *Helicobacter pylori* gastric adenocarcinoma may originate from circulating mesenchymal stem cell (MSC). MSCs are believed to differentiate into gastric epithelial cells to contribute to the regeneration of the damaged gastric epithelium. The role of *H. pylori* in the cancer development and especially its impact on these particular target cells is unknown. *H. pylori* adherence on MSC could be the first step to impact on MSC differentiation.

Aim. To assess the adherence abilities of different *H. pylori* strains on murine MSC in vitro and investigate their effects.

Material and Methods. Six *bab*A1- or *bab*A2-positive *H. pylori* strains were used. MSCs were obtained from bone marrow of C57Bl/6 mice. MSCs were cultured on coverslips, and bacteria were added after 24 hours at a MOI of 300. Adherence was estimated by immunocytochemistry after 48 hours of coculture, and cellular morphologic changes were examined by microscopy.

Results. The *bab*A2 + J99, TN2GF4, and 43504 were more adherent (more than 10 bacteria per cells) than the *bab*A1 X472AL, 26695, SS1 (less than 5 bacteria per cells). The *cag*A or *vac*A status was not implied in adherence. Morphologic changes of MSC were not observed with any strain.

Conclusion. *H. pylori* strains are able to adhere on MSC. BabA is known to be an adhesin specific of Lewis b antigen. This antigen is present on gastric epithelium but its expression is not estimated on MSC.

Our study shows that bacteria could adhere on MSC, which could be a crucial step in interfering with their differentiation.

Abstract no.: P026
Construction of *Helicobacter pylori* γ-Glutamyl Transpeptidase Isogenic Mutants

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The association of *Helicobacter pylori* and various gastroduodenal diseases has been well established. However, the exact mechanisms of pathogenesis are poorly understood. γ-Glutamyl transpeptidase (GGT) has been proposed as one of the virulence factors of *H. pylori*. It is a 60-kDa protein comprising a large and a small subunit. Three different isogenic mutants of *H. pylori* were constructed to characterize the individual subunits. The first was an isogenic mutant lacking the full length *ggt* gene, whereas the second and third lacked the sequences coding for the large and small subunits of GGT, respectively. A comparative study was carried out by infecting AGS cells in vitro with wild type *H. pylori* and the various isogenic mutants. The apoptosis-inducing activity of all three mutants as shown by flow cytometry was significantly lower compared to the parental strain. Among the three mutants, there was no significant difference observed in their apoptosis-inducing activities. This suggests that both the large and the small subunit of GGT are important in inducing apoptosis in AGS cells. Further characterization of both subunits of GGT is currently being carried out to gain a better understanding of their roles in the pathogenesis of *H. pylori*.

Abstract no.: P027
Virulence Factors in an Irish *Helicobacter pylori* Isolate from Intestinal Metaplasia

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Background. There is continuing interest in identifying *Helicobacter pylori* virulence-associated genes that might predict the risk for symptomatic clinical outcomes. Numerous studies have reported worldwide variations in genotyping of *H. pylori* and the incidence of gastric cancer. The most serious of these is gastric cancer. Gastric cancer develops through various stages from inflammation, atrophy, and intestinal metaplasia.

Aims. The aim of this study was to compare the virulence-factor associated genes (*cagA*, *vacA*, *babA2*) in *H. pylori* strains isolated from an Irish adult intestinal metaplasia (IM) and chronic gastritis (CG) group patients.

Methods. DNA was extracted from 80 *H. pylori* strains obtained from patients with either IM or CG. *cagA*, *vacA*, and *babA2* amplification were identified by polymerase chain reaction (PCR).

Results. The *cagA* were identified in 55% of *H. pylori* strains. The *vacA* gene was amplified in all of 80 strains, 23% containing bacteria carrying the subtype s1m1 53% in the IM strains versus 0% in chronic gastritis 23% contained bacteria carrying the subtype *s1m1* 13% of *vacAs1m2* (29% versus 0%), and 65% of *vacAs2m2* (29% versus 0%) (100% versus 18%).

Conclusion. *H. pylori* possessing the virulence determinants *cagA, vacAs1m1,* and *babA2* may increase the risk for the development of precancerous lesions in a Irish population.

Abstract no.: P028
Can *dupA* Gene be Accepted as an Informative Marker of Duodenal Ulcer?

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Helicobacter pylori plays an important role in the pathogenesis of peptic ulcer disease. Recently, *dupA,* duodenal ulcer-promoting gene A, was identified in the plasticity region of *H. pylori.* It is composed of two genes, *jhp0917* and *jhp0918;* its presence was reported as a marker of increased duodenal ulcer and decreased gastric atrophy and cancer.

The aim of this study was to investigate the prevalence of *jhp0917* and *jhp0918* in various gastric and duodenal diseases in which *H. pylori* was isolated.

Materials and Methods. Stock cultures of 93 patients (72 paediatric and 21 adult patients) were included in the study. The *dup A* gene was amplified by using the primers JHP917 and JHP918.

Results. Of the 93 isolates, 31 possessed both *jhp0917* and *jhp0918* genes. Eight isolates were positive for *jhp0917* and 3 were positive for *jhp0918* only. The distribution of *jhp0917* and *jhp0918* among different gastroduodenal diseases are presented in Table 1.

Conclusion. Regarding these results, *dupA* gene cannot be considered as an informative marker for duodenal ulcer. These are the preliminary results of an ongoing study. For a definite result, more strains will be tested.

Table 1 The distribution of *jhp0917* and *jhp0918* among different gastroduodenal diseases

	jhp0917 (+) *jhp0918* (+)	*jhp0917* (–) *jhp0918* (–)	*jhp0917* (+) *jhp0918* (–)	*jhp0917* (–) *jhp0918* (+)
Antral nodularity	11	27	3	0
Duodenal ulcer	5	7	1	0
Gastritis	3	6	4	2
Other diseases	3	5	0	0
Normal endoscopic findings	9	6	0	1

Abstract no.: P029
Interleukin-1 Polymorphisms and Gastric Mucosal Cytokines in Gastric Cancer at an Early Age under 50 Years Compared to the Older Age Group

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Background. *Helicobacter pylori* infection has been accepted as etiologically important in the development of gastric cancer. An outcome of *H. pylori* infection may be related to the interaction of bacterial factors and host inflammatory responses, which is partly governed by polymorphisms in proinflammatory genes.

Methods. Gastric biopsies were obtained from 52 gastric cancer patients (17 patients at age = 50 years, 35 patients at age > 50 years) and the antral mucosa from 51 patients with gastritis. Cytokines were measured from supernatants of biopsy homogenates using enzyme-linked immunosorbent assay. Cytokine levels and host IL-1 polymorphisms were compared between diseases.

Results. The prevalence of *H. pylori* infection was significantly higher in patients with gastric cancer age = 50 years (100%) or gastric cancer age > 50 (91.2%) than those with gastritis (47%). All mucosal IL-1β, IL-6, and IL-8 levels (pg/mg protein) were significantly higher among gastric cancer patients age = 50 years or gastric cancer patients age > 50 compared with gastritis patients (such as 2106 versus 1030 versus 133 pg/mg protein for IL-1β) ($p < .05$). However, we did not find any association between clinical outcomes and IL-1 genotypes for both Il-1β-511 and Il-1 RN genotypes.

Conclusions. *H. pylori* infection was found in all gastric cancer patients age < 50 years in Thailand. Mucosal cytokine levels in gastric cancer patients were significantly higher than gastritis and were related to *H. pylori* infection. Both Il-1β-511 and Il-1 RN genotypes did not associate with gastric cancer development in Thailand.

Abstract no.: P030
The Value of Serum Recognition of the *Helicobacter pylori* CagA Protein in Patients up to 45 Years of Age before Endoscopy

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Aim. to assess the diagnostic value of pre-endoscopy anti-CagA serum screening of patients up to 45 years of age with uncomplicated simple dyspepsia.

Methods. In a group of 207 dyspeptic patients (excluding patients with alarm symptoms or earlier eradication therapy) *Helicobacter pylori* status was determined by histology, microbiology,

and serology. Serum recognition of *H. pylori* anti-CagA antibodies was determined by the Western blot method.
Results. Of 207 (range: 18–88 years) *H. pylori*-positive patients, 58/207 were younger or equal and 149 older than 45 years of age. The sensitivity and specificity of Western blot in determining *H. pylori* status was 100% (gold standard microbiology and histology). Endoscopic findings in 45/58 dyspeptic patients younger than 45 years (77.5%) were significant gastroduodenal lesions (ulcers, cancer, or severe histology changes – atrophy or intestinal metaplasia). Screening strategies based solely on serum recognition of the CagA protein would have detected 91.7% of significant lesions. Unfortunately, one carcinoma and three duodenal ulcers would be missed. Screening by CagA serology plus anamnesis of NSAIL intake would not increase percentage of detected severe lesions.
Conclusions. In *H. pylori*-positive patients (detected by noninvasive methods) with uncomplicated simple dyspepsia up to 45 years of age, additional screening for endoscopy solely by CagA serology identified 91.7% of significant gastroduodenal lesions. Therefore, the results of this noninvasive screening test is not recommended as the only selective criteria for endoscopy in *H. pylori* positive dyspeptic patients up to 45 years of age.

Abstract no.: P031
Virulence Gene Expresión during *Helicobacter pylori*–Host Interactions

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Helicobacter pylori infection induces chronic gastritis, ulcers, and is a risk factor for gastric cancer. The *H. pylori* cag pathogenicity island (cagPAI) contents ~27 ORFs that encode a type IV secretion system through which CagA is injected into epithelial cells inducing cytoskeletal rearrangements and activation of transcription factors. Its presence is associated with more severe outcome. Little is known about in vivo expression of the cagPAI genes in different pathologies. We aimed to standardize a method to evaluate relative gene expression by real time reverse transcriptase–polymerase chain reaction (RT-PCR) in the human gastric mucosa.
Methods. Primers were designed for each cagPAI gene using OLIGO 6.0 software and *H. pylori* 26695 and J99 genome sequences. Tm values were between 68 °C and 70 °C and yielded PCR products ~250 bp. Primer efficiency was evaluated using *H. pylori* DNA and we selected only those with 85% efficiency or higher. Bacterial RNA from culture was extracted using the trizol method and we performed RT-PCR using rTth DNA polymerase. We compared relative gene expression using the 16s gene as reference. Once the method was standardized in vitro we next applied the system using total RNA from infected human gastric biopsies.
Results. All cagPAI genes were amplified and quantitated except cag2. For some genes, two or more primer pairs were designed. When we used RNA from two human gastric biopsies (gastritis and cancer), most of the genes could be evaluated obtaining interesting results; proving that the method is useful to evaluate *H. pylori* gene expression in vivo.

Abstract no.: P032
Significance of cagA Status and vacA Subtypes *of Helicobacter pylori* in Determining Gastric Histopathology in Pakistan

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Background/Aims. Early studies have suggested that the presence of cagA and vacA genes were virulence factor for *Helicobacter pylori*. We aimed to investigate the clinical significance of tissue cagA and vacA allelic status in *H. pylori*-infected patients and to assess its association with histologic changes in the gastric mucosa.
Methodology. Two hundred and forty patients with *H. pylori* infection established by both urease test and histologic examination were included in the study. The symptoms of the patients were recorded, and biopsies (antrum and body) were evaluated histologically according to the Sydney system. The cagA and vacA allelic status was determined by polymerase chain reaction.
Results. One hundred and twenty-five patients (52%) were infected with cagA-positive strains of *H. pylori*. The positivity of vacA allele s1a was 160(67%), s1b 79 (33%), m1121 (50%), and m2114 (47%). The distribution of CagAvacs1a and m1 was 55 (30%), CagAvacs1b and m1 26 (11%), CagAvacs1a and m2 30 (12.5%), CagAvacs1b and m2 16 (7%). The *H. pylori* density was significantly associated with inflammation activity $p = .005$, metaplasia $p \leq .001$, involvement of specialized and nonspecialized mucosa $p < .001$, and neutrophil infiltration $p < .001$. CagAvacs1a and m1 and CagAvacs1b and m1 had a borderline significance associated with inflammation activity $p = .06$ and $p = .06$, respectively. CagAvacs1a and m1 and CagAvacs1b and m1 were significantly associated with neutrophil infiltration $p = .01$ and $p = .002$, respectively, and metaplasia $p < .001$ and $p = .001$, respectively.
Conclusion. The virulence markers CagAvacs1a and m1 and CagAvacs1b and m1 of *H. pylori* are associated with gastroduodenal diseases in Pakistan. However, the distribution of CagAvacs1a and m1 and CagAvacs1b and m1 associated with florid gastroduodenal diseases is not marked locally as compared to in Western population.

Abstract no.: P033
Urease Activity and Urea Gene Sequencing of Coccoid Forms of *Helicobacter pylori* Induced by Different Factors

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Urease activity of *Helicobacter pylori* is an important maintenance factor. Determination of urease activity and possible mutations in the DNA sequences of coccoid bacteria will supplement to the understanding of pathogenesis of infections, which these forms are responsible.

In this study, our aim was to analyze the urease activity and investigate the urease gene sequences of cocoid *H. pylori* forms induced by different factors with respect to the spiral form. For this purpose, the urease activities of *H. pylori* NCTC 11637

standard strain and two clinical isolates were examined before and after transformation of the cells to coccoid forms by different methods such as; exposure to amoxicillin, aerobiosis, cold starvation, and aging. The effects of these conditions on urease gene were examined by the amplification of 411 bp *ureA* gene and 115 bp *ureB* gene regions by polymerase chain reaction (PCR) and sequencing of the *ureA* gene. The urease activities of coccoid cells were found to be lower than the spiral form. *ureA* and *ureB* gene regions were amplified in all coccoid cells by PCR. Inducing the change to coccoid form by different methods was found to have no effect on the nucleotide sequence of the *ureA* gene. These results suggest that coccoid *H. pylori* also has a potential for pathogenicty and it may cause infection after converting to spiral form under appropriate conditions.

Abstract no.: P034
Virulence Markers (cagA, vacA) of *Helicobacter pylori* and Gastric Histopathology

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Helicobacter pylori is an important human pathogen associated with gastrointestinal diseases such as gastritis, gastric and duodenal ulcer (peptic ulcer disease), and gastric cancer. A number of pathogenic factors (cagA, vacA) have been described for this bacterium, and some of them have been proposed as markers for the prediction of the clinical outcome. The predominant *H. pylori* strains circulating among geographic locations differ in regard to genomic structure.

The aim of the present study was to analyze such an association of cagA presence and vacA s1 presence of *H. pylori* with histopathological findings in patients with gastritis.

Polymerase chain reaction was used to detect cagA and vacA genes of *H. pylori* using specific primers. Histologic assessments were divided into four groups: normal, chronic gastritis, active chronic gastritis, and chronic atrophic gastritis.

Of the 102 patients, 37 (36.3%) were cagA positive and 63 (61.8%) were vacA s1 positive. Among isolates from 27 patients with active chronic gastritis, 8 patients with chronic gastritis and 2 patients with normal gastric histopathology, 45.7%, 21.6% and 40.0%, respectively, were cagA positive ($p = .019$). Most (61.8%) isolates were typed as vacA s1.

In this study, patients with nonulcer dyspepsia had a frequency of cagA positivity similar to that of the overall population. vacA s1 of *H. pylori* appear to have no association with histopathologic findings of gastritis. There was a significantly positive correlation between cagA genes and active chronic gastritis.

Poster Round 1.4: Other *Helicobacters*

Abstract no.: P035
A Proteomics Investigation Revealed Differences in the Modulation of the Expression of *Helicobacter hepaticus* Virulence Determinants by Bovine, Porcine, and Human Bile

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Helicobacter hepaticus colonizes the intestine and the liver in mice causing hepatobiliary disorders such as hepatitis and hepatocellular carcinoma, and has been associated also with inflammatory bowel disease in children. To elucidate virulence and host-specific adaptation mechanisms of *H. hepaticus,* a proteomics study of its response to bovine, porcine, and human bile was performed.

H. hepaticus was grown for 48 hours in media supplemented with 0–5% bovine, porcine, or human bile. The effects of bile on growth were determined by measuring bacterial growth (cfu/mL) at 0 and 48 hours at different bile concentrations. The protein expression profiles of bacteria grown without bile were compared to those grown at sublethal bile concentrations of 0.1% bovine or porcine bile, and 0.05% human bile using two-dimensional gel electrophoresis. Proteins were identified by tandem mass spectrometry. Validation of results was carried out using real-time reverse transcriptase polymerase chain reaction. Functional classifications of proteins differentially expressed were performed employing the database for annotation, visualization and integrated discovery.

In bovine, porcine, or human bile, the bacterium modulated differently the expression of several virulence determinants including the cytolethal distending toxin (CDT), urease, superoxide dismutase, flagellin, and ferritin. For example, superoxide dismutase was down-regulated in the three types of bile; CDT was down-regulated in bovine and human bile, but was unaffected by porcine bile; urease was down-regulated in bovine bile, up-regulated in porcine bile, and unaffected in human bile. Overall, the data suggested that bile serves as an environmental cue for protein expression by *H. hepaticus.*

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Changes in the Expression of Virulence-Related Genes in Response to Ox Bile of Six Species of Campylobacterales

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The order Campylobacterales includes bacteria belonging to the genera *Arcobacter*, *Campylobacter*, *Helicobacter*, and *Wolinella.* These