studied the involvement of the T4SS in the activation of alternative NF-κB by infecting mice with bacteria lacking CagE, a key structural component of the T4SS. We observed activation of alternative NF-κB in gastric epithelial cells in vitro and in vivo upon *H. pylori* infection, which was dependent on a functional T4SS, but independent on the presence of CagA. Notably, activation of alternative NF-κB highly influenced the inflammatory response to *H. pylori*, contributing to gastric pathology induced by the bacterium. Together, our results suggest that T4SS-dependent activation of alternative NF-κB plays a major in the pathogenesis of *H. pylori* infection.

## P04.11 | Can bacterial virulence factors predict antibiotic resistant *Helicobacter pylori* infection?

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*ISG Abstract Summer 2016*

**Introduction**: Virulence factors (CagA and VacA) produced by *H. pylori* contribute to the pathogenicity of the organism. The frequency of virulence factor genotype differs worldwide and recent data suggest that the *cagA* and *vacA* genotype may influence treatment outcome.

**Aim**: To evaluate the impact of *cagA* and *vacA* genotype on the prevalence of primary *H. pylori* antibiotic resistance.

**Methods**: Following ethical approval and informed consent, DNA was isolated from gastric biopsies of treatment-naïve adult patients infected with *H. pylori* (determined by histology). Virulence factor genotyping was performed using PCR and genotypic susceptibility to clarithromycin and levofloxacin was tested using the GenoType HelicoDR assay (Hain Lifesciences). The chi-squared test was used to assess correlations between genotypes and resistance.

**Results**: 50 samples from *H. pylori* positive patients, average age 47.6 years, 56% male ($n$ = 28), were analysed. The distribution of virulence factor and rates of resistance genotype are shown in the table below. The rate of clarithromycin resistance was significantly higher among $cagA^-$-than $cagA^+$-patients (48.3% vs 21.1%, $\chi$ = 3.74, $p$ = 0.05, OR 0.2844). There was no significant difference in either the clarithromycin or levofloxacin resistance rate between *vacA* genotypes.

| Genotype | % (Number of strains) |
|---|---|
| *CagA* Status | |
| Positive | 38 ($n$ = 19) |
| Negative | 62 ($n$ = 31) |
| *VacA* Alelle | |
| S1/M2 | 36 ($n$ = 18) |
| S1/M1 | 34 ($n$ = 17) |
| S2/M2 | 28 ($n$ = 14) |
| S2/M1 | 2 ($n$ = 1) |
| Clarithromycin resistant | 38 ($n$ = 19) |
| Levofloxacin resistant | 6 ($n$ = 3) |
| Dual resistance | 6 ($n$ = 3) |

**Conclusions**: $CagA^-$ and *vacA* S1/M2 are the dominant genotypes in *H. pylori* strains in our cohort. Infection with $cagA^-$ *H. pylori* may predict clarithromycin resistance.

## P04.12 | Determination of biofilm formation by *Helicobacter pylori*

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**Background**: Certain *H. pylori* strains form biofilm in laboratory experiments and also on the surface of gastric mucosa, suggesting possible reason for eradication failure by increasing resistance to antimicrobial agents and transmission.

**Aim**: To examine the mature biofilm formation by *H. pylori* NCTC11637 standard strain in different incubation periods for *H. pylori* biofilm characterization.

**Methods**: *Helicobacter pylori* standard strain was cultured. Bacterial suspension ($6 \times 10^8$ CFU/mL) in Brucella Broth supplemented with 5% FBS was inoculated into six-well plate with sterile glass coverslips to allow adherence of *H. pylori* at the air-liquid interface, incubated in microaerophilic conditions at 37°C for 48, 72, 96 h with shaking. Biofilm formation was evaluated by Crystal viole (CV) staining and SEM analysis. Planctonic bacteria were removed, formed biofilm was dyed and determined at 595 nm with ethanol to measure amount of biofilm formation. The coverslips were prepared for SEM analysis including paraformaldehyde fixation, graded ethanol hydration and coated with gold.

**Results**: The biofilm formation was observed after 48 of incubation by SEM analyses, Gram preparation and CV quantitation

assay. There was an increased biofilm formation observed in the following 72 and 96 h of incubation. Avarage absorbances of samples (duplicates) for 48, 72 and 96 h incubation were 0.3, 0.5 and 0.9, respectively.SEM analyses and Gram staining were concordant to both our findings and literature data.

**Conclusions**: The mature biofilm was formed at the end of 72 h incubation. At present, biofilm formation by *H. pylori* has not been extensively characterized in standard *H. pylori* strains as well as clinical isolates. The ability of *H. pylori* strain to form biofilm was shown in this preliminary study and further studies should be done in order to characterize biofilm mechanisms.

## P04.13 | Genetic and functional analyses reveal high conservation and crucial role of *Helicobacter pylori* serine protease HtrA

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HtrA proteases and chaperones exhibit important roles in periplasmic protein quality control and stress responses.[1,2] Inactivation of *htrA* has been described for many bacterial pathogens. However, HtrA of *H. pylori* is secreted into the extracellular environment where it cleaves the tumor-suppressor E-cadherin interfering with gastric disease development, but *htrA* mutants are not yet available. Here, we show that the *htrA* gene locus is highly conserved in strains from all continents. *HtrA* presence was confirmed in >1000 isolates of infected patients from Europe, Africa, Asia, Australia and America. mRNA expression studies indicated that *htrA* is encoded in an operon with two subsequent genes, HP1020 and HP1021. Genetic mutagenesis and complementation studies revealed that HP1020 and HP1021, but not *htrA*, can be mutated. In addition, we demonstrate that inhibition of HtrA proteolytic activity is sufficient to kill *H. pylori*, but not other bacteria. We show that *Helicobacter htrA* is an essential bifunctional gene with crucial intracellular and extracellular functions. Thus, we describe here the first microbe in which *htrA* is an indispensable gene. HtrA can therefore be considered as a promising new target for anti-bacterial therapy.

[1]Tegtmeyer N, Moodley Y, Yamaoka Y, Pernitzsch SR, Schmidt V, Traverso FR, Schmidt TP, Rad R, Yeoh KG, Bow H, Torres J, Gerhard M, Schneider G, Wessler S, Backert S. (2016) Mol Microbiol. 99(5):925–44.

[2]Schmidt TP, Perna AM, Fugmann T, Böhm M, Jan Hiss, Haller S, Götz C, Tegtmeyer N, Hoy B, Rau TT, Neri D, Backert S, Schneider G, Wessler S. (2016) Sci Rep. 6:23264.

## P04.14 | Identification, molecular basis and function of *Helicobacter pylori* ITAM-Like GroELs associated with CagAs

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Diverse ITAM-like CagA proteins are important *Helicobacter pylori (Hp)* virulence factors. We found that *Hp*-GroEL, *Hp*-urease beta-subunit and *Hp*-OipA also have ITAM-like motifs. The details of moleculer-interactions between *Hp*-ITAM-like antigens (including *Hp*-CagAs, *Hp*-GroELs, *Hp*-urease subunits and *Hp*-OipAs) and T4SS-CagII components remain unclear. We used Western blot and LC/MS/MS to analyze the molecular interactions and translocations in *Hp*-infected AGS cells and analyzed by PCR with Virus-binding Viro-Adembeads (VA-beads) to detect *Hp*-periplsmic cDNAs (derived from *Hp*-bacteriophage). We investigated a VA-beads-bound 34 kDa OipA-like protein-band by LC/MS/MS. IL-8 and IL-6 productions were measured by ELISA. We found the presence of diverse 34 kDa functional OipA homologues in *Hp*-periplasm, *Hp*-cytoplasm and *Hp*-membrane fractions. PCR with OipA-primers detected two types of gene-loss-stress inducible *hp-gro*EL cDNAs (1641 bp and/or 828 bp) in *oip*A-knockout mutant bacteria. LC/MS/MS analysis of a VA-beads-bound 34 kDa OipA-like protein, cross-reacted with OipA-antisera, identified *H. pylori* GroEL chaperonin, which dominantly-possess N-terminal-peptide fragments of GroEL, together with *Hp*-urease subunits and diverse CagAs. *Hp*-urease subunits-associated ITAM-like GroELs including N-terminal GroEL are secreted with CagAs via OipA-related OMVs and via T4SS-CagII associated with OipA and OipA-related OMPs in the early-phase of *Hp*-infection, which were involved in *Hp*-induced IL-8 and IL-6 productions in T4SS-cagII components-dependent way. Our rabbit pY- and non-pY-ITAM-like-peptide pAbs and synthetic ITAM-like peptides were effective against neutralizing of virulent *Hp*-ITAM-like antigens and -peptides in IgG-dependent way. Our findings may shed light on understanding of the pathogenesis of functional *Hp*-ITAM-like