

	Nordm; strains/total (%)	Zone inhibition: 7–14 mm	Zone inhibition: 15–20 mm
Grape extract	7/25 (28%)	5	2
Grape seed extract	25/25 (100%)	10	15

Abstract no.: P1.05

PROLONGED PRIMARY INCUBATION IN THE ISOLATION OF *HELICOBACTER PYLORI* FROM A PATIENT WITH FAILED ERADICATION THERAPY: A CASE REPORT

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Helicobacter pylori, a gastric pathogen, is strongly associated with peptic ulcer disease, and is an important risk factor for the development of gastric malignancies. Culture of the bacterium from gastric biopsy is the “gold standard” in confirmation of the diagnosis of *H. pylori* infection, and is essential for determination of the drug resistance of *H. pylori*. However, primary isolation of *H. pylori* from gastric biopsies is rather demanding, and is affected widely by number of factors such as biopsy preparation, transport and culture media, and the method adopted. The duration of incubation for isolation of *H. pylori* has been recommended to be 5–7 days.

However, in the present case, we found that a prolonged incubation period of up to 14 days allowed successful isolation of *H. pylori* from a patient with an *H. pylori* positive duodenal ulcer who received triple therapy that failed to eradicate the bacterium. The biopsies were placed directly into transport medium and processed for culture within 2 hours. On day 14, some suspected *H. pylori*-like colony appeared on one of the plates. The isolate was confirmed to be *H. pylori* based on its typical colony morphology, negative Gram's stain, and positive urease, catalase, and oxidase tests. The isolate requiring 14 days recovery, later exhibited normal growth characteristics of *H. pylori* strains, indicating its unusually long incubation requirement was a temporary predicament.

Our report demonstrates that longer incubation time is needed for some strains, especially those enduring hostile environment or a period of antibiotic force.

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HELICOBACTER PYLORI INFECTION AND COLON DISBIOSIS: IS THERE A LINK?

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Objectives: Disorders of colon microbiota in patients infected with *Helicobacter pylori* are widely investigated. We decided to evaluate content of *Bifidobacteria*, *Lactobacilli*, *Enterococci* and *Candida albicans* in stool of *H. pylori* infected patients and answer on the question: is there a link between *H. pylori* and colon microorganisms?

Methods: One hundred and three persons infected with *H. pylori* were observed. For all patients gastroduodenoscopy with biopsies from a stomach body and antrum were performed for verification of *H. pylori* infection (rapid urease test, polymerase chain reaction and histological method). Amount of *H. pylori* was estimate in stomach body and antrum by evaluation quantity of microbes (microscopic analysis): <20 microbes - mild (1 grade), 20–50 - moderate (2 grade), >50 - high (3 grade). Bacteriological analysis of stool was performed to evaluate content of microorganisms in colon and degree of colon disbiosis (1–4 grades). Statistical analysis was performed in Statistica 6.0 for Windows XP.

Results: We build two models of multiple linear regression: (1) $Y_{HPb} = 1.539 - 0.280X_1 + 0.279X_2$, where Y_{HPb} - amount of *H. pylori* in stomach body (1–3 grades), X_1 - content of *Bifidobacteria* in colon, lgcfu/g, X_2 - content of *Candida albicans* in colon, lgcfu/g ($p < .05$). (2) $Y_{HPa} = 0.927 + 0.253X_1 + 0.161X_2$, where Y_{HPa} - amount of *H. pylori* in stomach antrum (1–3 grade), X_1 - content of *Candida albicans* in colon, lgcfu/g, X_2 - degree of colon disbiosis (1–4 grades) ($p < .05$).

Conclusion: The link between *H. pylori* and colon microbiota is possible. It is need to perform next studies to confirm it.

Abstract no.: P1.07

POSITIVE SELECTION IN THE EVOLUTION OF *HELICOBACTER PYLORI* OUTER MEMBRANE PROTEINS

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Homologous recombination in *Helicobacter pylori* has been extensively described to occur via Outer Membrane Proteins (OMPs), regulating protein expression and generating allelic diversity, while the importance of single nucleotide polymorphisms (SNP) remains little studied.

We used an OMP-encoding gene, *homC*, as a model to evaluate the weight of positive selection in the evolution of *H. pylori*, by using >200 sequences obtained from strains collected worldwide. N-site and branch-site phylogenetic analysis by maximum likelihood models were used to identify specific codons that may be important in *homC* evolution, and to evaluate the impact of selective pressure on the geographic segregation of strains, respectively.

The N-site overall analysis showed that 14 of the 742 (1.9%) *homC* codons are likely under positive selection (likelihood-ratio test (LRT), $p < 10^{-61}$). Four of these codons are located in the most variable allelic gene middle region, probably reflecting recombination-derived hitchhiking events. On the other hand, eight codons are located in the more conserved 5' and 3' gene regions, although the significance of this distribution remains to be clarified.

Branch-site analysis revealed 36 codons (4.9%) under positive selection (LRT, $p < 10^{-41}$), showing a non-random distribution, and 89% of these particular codons ($p < 10^{-3}$) support the phylogenetic segregation of European strains from both African and East Asian strains. The lack of visible recombination within this segment suggests an important biological role of point mutations in the evolution of *H. pylori* OMPs.

In conclusion, *homC* SNP analysis suggests that, besides recombination, positive selection contributes as well to the evolution of *H. pylori* OMPs.

Abstract no.: P1.08

ASSEMBLY COMPARISONS: RE-SEQUENCING OF THE *H. PYLORI* J99 AND 26695 STRAINS USING ION TORRENT AND ILLUMINA MISEQ NEXT GENERATION SEQUENCING TECHNOLOGIES

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Molecular epidemiology by whole genome sequencing is a rapidly growing and evolving area. Benchtop sequencing machines that produce millions of reads of short DNA sequences are becoming standard tools for laboratory analysis. We aimed to understand the accuracy of de novo genome assemblies derived from both the Ion Torrent and the MiSeq sequencing machines. We analysed the accuracy of each coding sequence (CDS) by re-sequencing and assembling the two completely sequenced and finished strains, J99 and 26695. We found that despite high quality data, the genome assemblies displayed limited accuracy and varying results. J99 was assembled more accurately than 26 695 by data derived by both machines. The number of coding sequences for that were 100% accurate in the J99 assemblies were 1028 (69%) for Ion Torrent and 1207 (81%) for MiSeq out of the annotated total of 1491. In 26695, the number of correct genes were substantially fewer with 693 (44%) for Ion Torrent and 965 (62%) for MiSeq out of 1566 annotated genes.

Abstract no.: P1.09

SINGLE NUCLEOTIDE POLYMORPHISMS IN PRO- AND ANTI-INFLAMMATORY CYTOKINES AND THE RISK OF GASTRIC CANCER IN IRAN

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Interleukins depending on their tumor promoting or suppressing functions are known to affect cancer risk. IL-2 and IL-4 are respectively known as pro and anti-inflammatory cytokines which are affected by *H. pylori* infection and involved in predisposition to gastric cancer. We have, herein, investigated the risk of gastric cancer associated with of IL-2 -384G/T and IL-4 -590C/T SNPs and its interaction with *H. pylori* infection.

Gastric cancer patients (N = 254) and healthy controls (N = 251) were evaluated for *H. pylori*-specific serum IgG antibodies by ELISA as well as IL-2 -384G/T and