Introduction:

*Helicobacter pylori*, originally isolated as a possible cause of gastritis and peptic ulceration in 1982, is still the subject of much controversy in the post-genomic era. This ubiquitous organism has probably infected mankind for millennia, so while we attempt to eradicate it in disease states, we must also look for ways in which it might confer an advantage to its host.
Taxonomy of Helicobacter spp by 16S RNA

There are many species of Helicobacter. This dendrogram shows the classification of Helicobacters based on 16S RNA sequencing. Notice Helicobacter pylori at the top of the chart and its closely related Helicobacter nemestrinae (Helicobacter from macaques).

Classification is: Bacteria $\rightarrow$ Proteobacteria $\rightarrow$ epsilon subdivision $\rightarrow$ Helicobacter group $\rightarrow$ Helicobacter $\rightarrow$ Helicobacter pylori
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Classification is: Bacteria → Proteobacteria → epsilon subdivision → Helicobacter group → Helicobacter → *Helicobacter pylori*
Microbiology of *H. pylori*

- Microaerophilic
  - (CO₂) Incubator or gas jar with 'campylobacter' atmosphere
  - 37°C for 3-6 days
- Gram Negative
  - Long rods with “U” shapes
  - Motile
- Catalase+, Oxidase+, Urease+
  - Urease reaction occurs in 5 minutes

**Culture of *H. pylori***

From a gastric biopsy, the specimen should be moistened with a drop of sterile saline and transported to the laboratory within 3 hours (*H. pylori* survives for up to 24 hours in a moist sample, longer if refrigerated). In the lab, the specimen may be minced or ground up. Part of the sample is used for gram stain, the remainder plated onto brain heart infusion blood agar (BHIB) or equivalent. The selective media such as Skirrows or BHIB with Dent supplement improve isolation rates.

Culture in a microaerophilic atmosphere (CO₂ incubator, "campy pak" or similar) for 3-6 days at 37°C. Note that some Helicobacters prefer 42°C e.g. *H. mustelae*.

Colonies appear as "water spray" type visible on day 3. Identify in gram stain where they are pleomorphic long rods and U shapes. A few cultured organisms have spiral shapes, mainly in liquid media.

Colonies are rapid urease positive (5 minutes) as well as oxidase and catalase positive.
J. Robin Warren’s initial observation was that these spiral bacteria not only colonised the gastric mucosa, but lived happily there in large numbers, and were associated with gastritis (**Warren JR. Lancet 1983 (letter)). Silver stains revealed masses of the organisms, particularly in the antral mucus-secreting glands. In the corpus mucosa, where acid secreting parietal cell glands predominate, the numbers of bacteria were less with organisms mostly in the superficial layers of the gland necks. Similarly, in the proximal the stomach, the inflammation tended to also be superficial, perhaps explaining how the term “superficial gastritis” was coined many years earlier, probably on the basis of blind suction biopsies which tended to sample this area of the stomach rather than the antrum. The silver stain is the best way to demonstrate bacteria in the gastric mucosa because the metal precipitates on the organisms and makes them slightly larger than their original size, while leaving the tissues relatively pale.
In routine practice, a simple toluidine blue stain, or a Giemsa stain, allows histological sections to be automatically processed so that *Helicobacter pylori* can be reported at the same time as the Haematoxylin and Eosin (H&E) section, with little extra cost.
Normal Gastric Mucosa
One thing is certain, H.pylori are always associated with a degree of histological gastritis. Most of the inflammatory cells are mononuclear, but collections of polymorphonuclear leucocytes can be seen associated with the necks of the antral glands. In some cases the inflammatory lesion is slightly patchy, so that H.pylori may be found attached to normal mucosa. In every case however, further biopsy samples will always reveal gastritis nearby (**Bayerdorffer see Marshall helicobacter book from 1990). The presence of polymorphs in the mucosa is not called “acute” inflammation because the lesion is not new. Persons with active chronic gastritis have usually had the infection for many years thus explaining use of the term “active” to indicate PMN infiltration.
The polymorphs can also be seen in Gram stains of gastric mucus, and sometimes show numerous phagocytosed Gram-negative organisms. All these histological features are strong evidence that the human host sees *Helicobacter pylori* as a pathogen.

To emphasize the fact that the histological changes of what we call gastritis are normal accompanyment to the mucosal infection, immunological stains reveal that many of these cells are immunocompetent antibody producers, and that appropriate IgM/IgG/IgA antibody production can be detected in cultured gastric tissue. Because of this, IgG antibody specific to *Helicobacter pylori* remains as the most widely used non-invasive test for the organism. Unfortunately, antibody falls slowly after treatment so it cannot be easily used after therapy to confirm cure. In this role, more specific tests are preferred such as the urea breath test (**Pytest am j gastro 1997).
Peptic Ulcer
“An ulcer of the G.I. Tract in an area where acid is present”
i.e. the stomach and proximal duodenum
In many cases of H. pylori infection, the mucosa appears normal or only slightly irregular.

Antral “gooseflesh” also called “chicken skin”

The association between H. pylori and peptic ulcer is well known, but in many persons the endoscopy is normal. Peptic ulcer disease is a condition characterised by remissions and relapses so that the ulcer crater is not always present at endoscopy. One visible lesion found to be the best predictor of gastritis is the cobblestone, “gooseflesh” or “chicken skin” appearance of the antral mucosa. Other appearances such as redness remain for many years after treatment of the gastritis so they cannot be used as indicators of active Helicobacter infection.

The impossibility of endoscopic diagnosis for H. pylori resulted in the development of the biopsy rapid urease test “CLOtest” so that gastroenterologists could diagnose the infection with little effort in the endoscopy room (** CLOtest paper Am J Gastro 1997).
**Gastric Helicobacters and Urease**

Gastric Helicobacters are remarkable in their ability to produce urease. Urease enzyme, as shown here, splits urea, consuming a hydrogen ion to generate ammonia and bicarbonate. About 10% of the dry weight of *Helicobacter pylori* protein is urease, emphasizing the extreme importance of this enzyme. The organism probably maintains a microenvironment within the bacterial cell with a pH of 5 to 8. It has been shown that the cell is viable and continues to manufacture proteins within this range, even when the pH outside the cell is much lower, i.e. pH 2 to 5. For this acid protection mechanism to work however, urea must be present in the environment. Luckily, saliva, gastric juice and extracellular fluid contain urea in a concentration of at least 1 millimole per litre, which is sufficient for *H. pylori* to survive long enough for it to colonise the gastric mucus layer. Once below the gastric mucus, *H. pylori* is protected from acid, and the environmental pH is probably closer to 6.
Early Rapid Urease Tests for Hp
Initial Diagnosis of Hp

Two Biopsies:
1. Urease Test
2. Extra

Culture rate > 90%

60 min
14C-urea Breath Test

Time=0

Time=10
Starch granule

Mucus

C14-urea coating

mucosa

\[ \text{CO}_2 \]

\[ \text{NH}_3 \]

\[ \text{H}^+ \]

\[ \text{H}^+ \]

\[ \text{H}^+ \]

\[ \text{H}^+ \]

\[ \text{H}^+ \]

\[ \text{pH}=2 \]

\[ \text{pH}=6 \]

\[ \text{C}=\text{O} \]

\[ \text{NH}_2 \]
## Annual Natural Radiation

<table>
<thead>
<tr>
<th>Source</th>
<th>Annual Dose [µSv]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Your own C$^{14}$</td>
<td>50</td>
</tr>
<tr>
<td>Your own K$^{40}$</td>
<td>400</td>
</tr>
<tr>
<td>Your spouse (K$^{40}$)</td>
<td>15</td>
</tr>
<tr>
<td>UBT</td>
<td>3</td>
</tr>
<tr>
<td>Plane travel 2hrs</td>
<td>6</td>
</tr>
</tbody>
</table>
Faecal Antigen Test
Diagnosis of HP

<table>
<thead>
<tr>
<th>%</th>
<th>Serology IgG</th>
<th>Urea Breath test</th>
<th>Faeces Antigen</th>
<th>Urease test</th>
<th>Culture Main Lab</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>95</td>
<td>93</td>
<td>98</td>
<td>75*</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>65</td>
<td>98</td>
<td>95</td>
<td>100</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>67</td>
<td>98</td>
<td>95</td>
<td>99</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>NPV</td>
<td>95</td>
<td>95</td>
<td>90</td>
<td>85</td>
<td>98</td>
<td></td>
</tr>
</tbody>
</table>

Simple: No preparation, GP's like it

Fasting: Easy to order

"Ulcer Patients":

Endoscopy Biopsy, 1 hour, unrestricted:
- Fasting: $25
- "Ulcer Patients": $65

Endoscopy Biopsy, 1 week, unrestricted:
- Fasting: $80
- "Ulcer Patients": $55

Endoscopy Biopsy, 2 days, unrestricted:
- Fasting: $100
- "Ulcer Patients": $75

Sensitivity: 95%, 95%, 93%, 98%, 75%
Specificity: 85%, 98%, 95%, 99%, 100%
PPV: 67%, 98%, 95%, 99%, 100%
NPV: 95%, 95%, 90%, 98%, 85%
In areas with low prevalence, a test with low specificity tends to over-diagnose the disease.
% infected

Developing

Western

Incidence 20%
Loss rate 3%

Incidence 2%
Loss rate 3%

age
Stable infection after childhood

“Cohort” of older persons has Hp

Developing Western

Stable infection after childhood

“Cohort” of older persons has Hp

Western
Age Related Prevalence of *H. pylori* in a Developed Country

Data from a paper describing the age-related prevalence of *H. pylori* in a randomly selected Caucasian population from Melbourne. Notice that infection rate was low in young adults (18-24%) but rose with age to be 50% in elderly persons. This study also noted that men were more likely to be infected than women (48% vs 30%).

With a different serologic test, lower rates of infection were observed in sera from West Australians in Busselton, who were bled in 1994. That data is shown by the dotted line.

Overall, about 30% of the total adult population in developed countries is thought to be infected with *H. pylori* versus 75% approximately in most developing countries.
Guilt by association is strong evidence that H. pylori is a pathogen. Numerous studies have shown variants on the association with peptic ulcer, gastric cancer, and gastric lymphoma. In any developed country, about 30% of the population are infected with *Helicobacter pylori* (HP). It is within this group that peptic ulcer disease develops. The great majority of duodenal ulcers and gastric ulcers are in infected patients. Although the majority of persons with Hp. Are asymptomatic at any point in time, many of them eventually develop disease. In prospective studies, the conversion rate to active peptic ulcer is about 1% per annum (**Cullen Busselton study**). Thus, during a lifetime, about one third of infected persons develop symptomatic disease. In developing countries the majority of persons (50-90%) may be infected with Hp. Although ulcer disease is usually quite common, its expression may be modulated by the degree of acid secretion possible in the host. Whereas in developed countries like the USA, peak acid output is likely to be 30 mEq per hour, values of 17 mEq per hour are usual in developing countries. Some of this may be nutritional because acid secretion in H. pylori-negative adults in Japan has been documented to have increased by 50% in the 25 years after 1970 (**Uemura and Haruma see clinics chapters**). Additionally, lifelong gastritis acquired in early childhood may impair infected persons from ever reaching adult levels of acid secretion. Thus, by damaging acid secretion, Hp may help produce the carrier state of asymptomatic gastritis. As an aside, the asymptomatic low acid state appears to be more likely in persons with a
particular makeup of interleukin polymorphisms, such as mucosal inflammation is enhanced (** El-Omar Nature 2000).
One puzzle with duodenal ulcer (the most common peptic ulcer in developed countries) was that the Helicobacter were present in the stomach, but the ulcer was in the duodenum. Biopsy analysis of duodenal biopsies from normal persons however has shown that islands of gastric mucosa are usually present in the duodenum, and that these are foci of infection, with severe PMN infiltration, in persons with Hp. Thus, the peptic ulcer may be initiated by the host’s inflammatory cells as much as by acid secretion or the bacteria themselves.
**Gastric Ulcer**

**Corpus**: Extensive, heavy, \( H.pylori \) colonisation of both antrum and body mucosa with severe pan-gastritis.

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High power P.A.S. section of duodenal mucosa from a patient with past duodenal ulcer. The patch of gastric metaplasia (GM) is easily identified by the deep pink staining of epithelial cells. In contrast, duodenal mucosa has a delicate, weakly staining brush border (BB) and goblet cells can also be seen (GC).
In active duodenal ulcer disease (with duodenitis) it can be seen that neutrophil infiltration is selective for glands with gastric metaplasia [*] whereas glands with normal duodenal mucosa (to which H. pylori cannot attach) [§] are spared.
As well as the histological appearance of Hp gastritis, its ability to generate ammonia in the gastric mucus is also a definite pathogenic factor. The TCA cycle is impaired when alphaketoglutarate is shunted towards glutamine in the presence of free ammonia. Thus ATP production is impaired in aerobic cells. In addition, ammonia, in oxygen free radicals and chloride (produced by inflammatory cells such as PMN’s) can form monochloramine, a carcinogen. Thus, as mentioned below, the inflammatory response itself can probably cause cancer, and this effect is likely to be enhanced in the more inflammatory types of helicobacter, such as those which carry the CagA pathogenicity island (** covacci review science 1999-2002).
Clinical studies will just be mentioned briefly here. Many groups have confirmed the cure of peptic ulcer in persons cured of their Helicobacter infection. Prior to antibiotic therapy, the H2 receptor blockers could heal ulcers but almost always the ulcer relapsed after therapy was ceased. The definitive study by Hentschel et al in 1992 (** NEJM) showed that relapse was rare in patients given ranitidine with amoxicillin and metronidazole whereas patients given ranitidine with placebo almost all relapsed. This therapy has since been replaced by the triple combination therapies which add a proton-pump inhibitor such as omeprazole to a double antibiotic therapy with clarithromycin and amoxicillin (** machII study ??NEJM). Combined with non-invasive testing using the urea breath test, Hp disease can now be managed easily by most general practitioners.
The success of Hp eradication as a means of treating peptic ulcer disease is not solely due to healing of the gastritis. Abnormal acid secretion states predominate in the presence of antral gastritis. Normally, acid secretion is regulated by a feedback loop involving the antral G-cell, the parietal cell, and the Somatostatin secreting D-cell.

Gastrin stimulates acid secretion, the stomach lumen is acidified, the D-cell senses this, it secretes somatostatin locally, the G-cell is inhibited, gastrin secretion ceases and acid secretion stops for a time. In this way the stomach pH is kept very low, but large amounts of acid are not continually secreted into the duodenum.

When Hp causes antral gastritis, secretion from the D-cell is impaired. This allows the G-cell to be rather uninhibited so that basal acid hypersecretion results. When Hp is cured, this hypersecretion gradually improves as the gastritis heals. This effect means that treatment of Hp in ulcer disease gives acid reduction, as well as healing the mucosa (**McColl acid basal hypersecretion).
The most sinister indicator of the pathogenic role of Hp is its confirmed link to gastric cancer. Foreman et al showed a consistent relationship between prevalence of Hp infection and incidence of gastric cancer in many countries (Figure) (** foreman ??BMJ 1979).
More recently, Koch’s postulates have been fulfilled for gastric cancer and Hp, in an animal model using the Mongolian gerbil. These animals are easily colonized by Hp and develop severe gastritis. In a study using 65 infected gerbils and some controls, 5/5 sacrificed were found to have gastric ulcer at six months, verses no controls. At twelve months, 37% of 47 infected animals had adenocarcinoma of the stomach (** watanabe gastro 1998 642-8). Thus there is little doubt that helicobacter infection causes stomach cancer.

This model opens the exciting prospect of the fullfillment of “molecular” Koch’s postulates for various Hp genes. It is now possible, with selective knockout experiments, to delete various putative cancer genes. It is then possible to see if such deletions modify the carcinogenic effect of Hp in the infected gerbil.
Can Eradication of *H. pylori* Prevent Gastric Cancer?

- 100 Early Gastric Cancers
- Endoscopic Resection
- Treatment of HP in Half the Patients

50 HP pos.
6 new cancers

50 HP neg.
0 new cancers

Uemura et al. AGA 1996

= YES IT CAN
** heather please check that the author is correct – is uemura a co-author of koike? I thought this was a uemura paper in NEJM.**

Same for next slide
Other Associated Diseases Have Been Reported (but not proven)

- Cardiovascular disease (MI)
- Growth retardation
- Rendaud’s phenomenon
- Migraine headaches
- Parkinson’s disease
- Idiopathic Thrombocytopenic Purpura

Rare complications of a common disease are hard to prove as associations with the disease rather than with socioeconomic and lifestyle factors.
- cagA gene group is a "pathogenicity island" responsible for delivery of HP toxin onto (or into) the epithelial cells.
- picB resembles B.pertussis secretion protein PtlC
Localization of gene products is based on homology with genes in *Agrobacter tumefaciens*. 
Action of Cag Pathogenicity Island

- Type IV secretion system
- Delivers CagA
  - Tyrosine phosphorylated
  - Growth factor activation
- Altered cell morphology
  - Spreading of cell
  - Improved attachment of Hp
Vac A

Action of vacA Toxin

urea

vacuoles
**H. pylori genome**

- ~1.7 mB depending on strain
- ~1600 genes ➔ relatively simple organism
- ~55% have homologues in other organisms
- ~45% currently unique to *H. pylori*
  - ~10% have partial structural & functional identifiers
  - ~35% have unknown functions
Bacterial Effects: Gene Arrays

A Comparative Hybridization Experiment

Ulcet vs. Cancer vs. No disease
Symptomatic Reflux as a Risk Factor for Esophageal Adenocarcinoma

<table>
<thead>
<tr>
<th>Heartburn, reflux, or both, at least once per week</th>
<th>Controls (N=820)</th>
<th>Esophageal Adeno CA (N=189)</th>
<th>Adeno CA Gastric Cardia (N=252)</th>
<th>Esophageal Squamous CA (N=167)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heartburn, reflux, or both, at night, at least once per week</td>
<td>135 (16%)</td>
<td>60% (7.7 (5-11))</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>66 (8%)</td>
<td>53% (10.8 (7-17))</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RR

Esophageal Adeno CA

0.9 (0.4-2.0)

2.4 (1.5-3.8)

Esophageal Squamous CA

1.1 (0.7-1.9)

2.0 (1.4-2.9)

There is a strong and probably causal relationship between reflux and oesophageal adenocarcinoma. Lagergren J.

The association is strong, dose-dependant, and plausible.
Is There a “Down Side” to Hp Eradication?

- CagA+ strains protect from:
  - Reflux
  - Adeno-Ca. Lower Oesophagus

- What is worse?
  - Gastric Ca risk Hp+
    - 7-50/100k/year
  - Oesophageal Ca risk Hp-
    - 4/100k/year
H. pylori OMPs

Hop family
(24)

Other OMP
(27)
“Genes to vaccine” process

Having identified protein antigens, have to show:

• Presence in all strains of *H. pylori*
  – (the “essential” genome of H.p. is about 750 genes)

• Surface exposed regions antigenically conserved

• Protective *in vivo* - initially in mice

• Formulate into vaccine
## Virulence Factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Function</th>
<th>Distribution</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urease</td>
<td>Buffers stomach acid</td>
<td>All strains</td>
<td>(37)</td>
</tr>
<tr>
<td>HopZ</td>
<td>Predatory</td>
<td>All strains</td>
<td>(52)</td>
</tr>
<tr>
<td>NapA</td>
<td>Neutrophil activation</td>
<td>All strains</td>
<td>(20)</td>
</tr>
<tr>
<td>BslA</td>
<td>Activates for E.</td>
<td>Prevalent on type I</td>
<td>(18)</td>
</tr>
<tr>
<td>LPS</td>
<td>Low toxicity</td>
<td>All strains</td>
<td>(63)</td>
</tr>
<tr>
<td>Lewis xy9</td>
<td>Molecular mimicry</td>
<td>Some strains</td>
<td>(19)</td>
</tr>
<tr>
<td>IceA</td>
<td>Homology of Nis III restriction endonuclease</td>
<td>Some strains</td>
<td>(54)</td>
</tr>
<tr>
<td>VacA</td>
<td>Cytotoxicity (two alleles)</td>
<td>All strains</td>
<td>(27, 23)</td>
</tr>
<tr>
<td>cag PAI</td>
<td>31 genes coding for type IV secretion system</td>
<td>Type I strains</td>
<td>(26)</td>
</tr>
<tr>
<td>CagA</td>
<td>Immuno dominant antigen</td>
<td>Type I strains</td>
<td>(33)</td>
</tr>
<tr>
<td>PicB</td>
<td>Equivalent to CagE</td>
<td>Type I</td>
<td>(26)</td>
</tr>
</tbody>
</table>
Typical Vaccine Experiment for *H. pylori*
Vaccine: Difficulties and Timeline

- Immune response varies in mouse strain used
- Antibodies to HP sometimes react with host tissues
  - autoantibodies may be associated with gastritis
- Timeline
  - 1999: volunteer studies in human infection (Houston)
  - 2000-2: phase 1 studies (healthy normal persons)
  - 2002-3: phase 2 studies (patients)
  - 2003-7: long term studies on children?
**Redundancy in the Genetic Code**

- CGTA in triplets
- 64 combinations for 20 AA’s
- Mutations in third codon may be “synonymous”

GGU, GGC, GGA, GGG = glycine
Ancestral Africa 1
Ancestral Europe1 (AE1)
Ancestral East Asia
Ancestral Africa 2
Ancestral Europe2 (AE2)

Kimura 2 parameter diameter/distance 0.01

Putative Modern and Ancient Migration of H. pylori

- Crossing of Bering Strait >12,000 BP
- Colonial expansion from 500 BP
- Slaves trade 470-150 BP
- Bantu expansion 3,000 – 5,000 BP; Reaches South Africa 1,300 BP
- Arrival of agriculture in Europe 8,500 BP
- Arrival of Uralic Speakers in Europe? BP
- Polynesian migration 4,000-5,500 BP; Reaches New Zealand 1,000 BP

Arrows indicate specific migrations of human and H. pylori populations. BP, years before present.
Proportion of children infected with *H. pylori* according to infection of parents (Northern Italian farm community)

<table>
<thead>
<tr>
<th>Parental infection</th>
<th>No (%) of children infected*</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (both parents infected)</td>
<td>116/265 (44)</td>
<td>9.9 to 50.1</td>
</tr>
<tr>
<td>Group 2 (one parent infected)</td>
<td>75/256 (30)</td>
<td>8.8 to 36.0</td>
</tr>
<tr>
<td>Father infected</td>
<td>34/133 (25)</td>
<td>17.5 to 32.5</td>
</tr>
<tr>
<td><strong>Mother infected</strong></td>
<td><strong>41/123 (33)</strong></td>
<td><strong>24.5 to 41.5</strong></td>
</tr>
<tr>
<td>Group 3 (neither parent infected)</td>
<td>17/80 (21)</td>
<td>9 to 30.1</td>
</tr>
</tbody>
</table>

*Significant trend (χ² test, P<0.001)
The mode of transmission of H.p. is controversial. Some evidence exists for intrafamilial spread as shown here.
The mode of transmission of H.p. is controversial. In countries where the environment (water) is contaminated, family members and couples can all have different strains.
When two “tribes” have different strains, the potential exists for the strains from Race 1 to obliterate the strains from Race 2.
Swallowed String Test

- Capsule
- String pulled out
- Petri dish
AFLP ANALYSIS OF SPOUSES' *H. PYLORI* ISOLATES

3 isolates from each spouse showing:

A. Each spouse infected with a different strain
B. Both spouses infected with the same strain
C. One spouse infected simultaneously with 2 different strains
Please make sure that the string is only 90 cm long, and is removed before 1 hour. Otherwise weird things might happen!
Whenever H. pylori eradication programs are discussed the issue of cost-benefit arises. Whereas governments might argue that Hp eradication is hardly worthwhile when most of the population is infected, individuals themselves easily justify such therapy in order to protect themselves from gastric cancer. Helicobacter really lives outside the body, on the surface of the gastric mucosa. It creates an inflammatory process which leads to gastric redness in most persons, but nothing more. Thus, for many individuals it is a cosmetic disease, only of concern to gastroenterologists who can see it. This is similar to dandruff, a surface infection with a fungus, which causes a cosmetic problem but is largely asymptomatic. Persons who treat their dandruff however might spend USD$10 per month on various shampoos which could easily add up to USD$1000 over a lifetime. Thus, at least in a developed country, many persons would spend $1000 on non-curative therapy for a cosmetic problem. It seems very easy therefore, to justify far less expensive curative therapy for a proven carcinogen such as H. pylori.

Although more pressing issues are likely to consume health budgets in developing countries, we should encourage public health measures and education about Helicobacter pylori, as well as supporting the diagnosis of infection so that individuals can then make their own decisions about therapy.
Culture of *H. pylori*

From a gastric biopsy, the specimen should be moistened with a drop of sterile saline and transported to the laboratory within 3 hours (*H. pylori* survives for up to 24 hours in a moist sample, longer if refrigerated). In the lab, the specimen may be minced or ground up. Part of the sample is used for gram stain, the remainder plated onto brain heart infusion blood agar (BHIB) or equivalent. The selective media such as Skirrows or BHIB with Dent supplement improve isolation rates.

Culture in a microaerophilic atmosphere (CO2 incubator, "campy pak" or similar) for 3-6 days at 37°C. Note that some Helicobacters prefer 42°C e.g. *H. mustelae*.

Colonies appear as "water spray" type visible on day 3. Identify in gram stain where they are pleomorphic long rods and U shapes. A few cultured organisms have spiral shapes, mainly in liquid media.

Colonies are rapid urease positive (5 minutes) as well as oxidase and catalase positive.
What if we used *H. pylori* to deliver vaccines
END