What’s new with *Helicobacter pylori* testing?

**Presenter:**

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Disclosures

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Helicobacter pylori Bacteriology

- Slender, curved Gram-negative bacilli
- Urease
- Mucosa stomach
- Growth
  - Microaerophilic atmosphere, slow
- World Health Organization
  - Group 1 carcinogen (gastric carcinoma)
**Helicobacter pylori Clinical Presentation**

- Asymptomatic
- Peptic ulcer disease (gastric, duodenal)
- Nonulcer dyspepsia
- Gastric carcinoma
- Gastric mucosa-associated lymphoid tissue lymphoma
  - MALT lymphoma, MALToma, marginal zone B-cell lymphoma of MALT type

**Helicobacter pylori Indications for Testing**

- Active peptic ulcer disease or history of peptic ulcer disease (unless *H. pylori* eradicated)
- Low-grade gastric mucosa–associated lymphoid tissue lymphoma or history of endoscopic resection of early gastric cancer
- Uninvestigated dyspepsia
- Long-term NSAID or aspirin use
- Unexplained iron-deficiency anemia after evaluation for other causes
- Immune thrombocytopenia in adults
**Helicobacter pylori Treatment**

- Typically treated with combinations of 2–3 antibiotics along with a PPI
- “...initial course of eradication therapy...generally offers the greatest likelihood of treatment success. Thus, careful attention to the selection of the most appropriate first-line eradication therapy for an individual patient is essential.”
- “The main determinants of successful *H. pylori* eradication are the choice of regimen, the patient’s adherence to a multi-drug regimen with frequent side-effects, and the [susceptibility] of the *H. pylori* strain to the combination of antibiotics administered.”

### Antimicrobial Susceptibility Test Results

**413 Helicobacter pylori Isolates, Mayo Clinic Laboratories**

<table>
<thead>
<tr>
<th>Amoxicillin (n = 410)</th>
<th>Ciprofloxacin (n = 409)</th>
<th>Clarithromycin (n = 412)</th>
<th>Metronidazole (n = 330)</th>
<th>Tetracycline (n = 409)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MIC</strong></td>
<td><strong>n</strong></td>
<td><strong>%</strong> **</td>
<td>** <strong>MIC</strong></td>
<td><strong>n</strong></td>
</tr>
<tr>
<td>≤2</td>
<td>405</td>
<td>98.8</td>
<td>≤1</td>
<td>190</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0.2</td>
<td>R</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>0.5</td>
<td>R</td>
<td>&gt;2</td>
</tr>
<tr>
<td>&gt;8</td>
<td>2</td>
<td>0.5</td>
<td>R</td>
<td></td>
</tr>
</tbody>
</table>

*CASI; **EUCAST; S, susceptible; I, intermediate; R, resistant; MIC, minimum inhibitory concentration (in μg/ml)*
Genetic Mechanisms of Clarithromycin Resistance

111 Helicobacter pylori Isolates, Mayo Clinic Laboratories³

• 23S ribosomal RNA gene sequencing → assess clarithromycin resistance-associated mutations
• Overall concordance phenotypic & genotypic results - 106 (95%) isolates
  • Resistant isolates - 88
    • A2143G - 70
    • A2142G - 12
    • A2142C - 3
    • Wild type - 3
  • Susceptible isolates - 23
    • A2143G - 2
    • Wild type - 21

Helicobacter pylori Diagnosis

• Non-invasive tests
  • Stool PCR – predicts H. pylori clarithromycin susceptibility or resistance
  • Stool antigen
  • Helicobacter pylori (urea) breath test
    • C¹³ or C¹⁴-labeled urea ingested
    • C¹³ or C¹⁴-labeled CO₂ detected
• Invasive tests - gastric biopsy
  • Rapid urease test
  • Stain of tissue
  • Culture (provides H. pylori isolate for amoxicillin, ciprofloxacin, clarithromycin, metronidazole and tetracycline susceptibility testing)
Helicobacter pylori with Clarithromycin Resistance Prediction, Molecular Detection, PCR, Feces

- 535 fecal specimens submitted for *H. pylori* stool antigen testing (Premier® Platinum HpSA® PLUS assay)
  - 524 analyzed (11 excluded due to inhibition)
    - 101 true positive, 13 false negative
      - Sensitivity 89% (95% CI: 81-94)
    - 398 true negative, 12 false positive
      - Specificity 97% (95% CI: 95-99)
  - Positive predictive value 89% (95% CI, 82-94)
  - Negative predictive value 97% (95% CI, 95-98)

- 113 PCR positive samples
  - 77 (68%) wild-type (clarithromycin susceptible)
  - 36 (32%) one of three clarithromycin resistance-associated SNPs
- Sanger sequencing 100% agreement
**Helicobacter pylori with Clarithromycin Resistance Prediction, Molecular Detection, PCR, Feces**

- 223 fecal samples 2015-2018 positive stool antigen
  - 197 unique Mayo Clinic patients
  - Retrospective review of electronic medical records
  - Demographic information, prior macrolide use, longitudinal data pertaining to diagnosis, treatment, follow up testing, treatment outcome
    - Treatment success or failure documented when test of eradication performed
      - Positive result = failure; negative result = success
    - *Initial diagnosis group* - mostly treatment-naive tested for *H. pylori* diagnosis. Some successfully treated *H. pylori*, or remote treated infection presenting with new positive diagnostic test considered a new episode.
    - *Test of eradication group* - tested for follow-up proof of eradication by antigen test after receiving antibiotic therapy for *H. pylori*

- Patient ages 3-91 years, most over 18 years
  - 139 female; 84 male
  - Clarithromycin triple therapy most frequently used initial therapy combination after *H. pylori* diagnosis (67%), followed by bismuth quadruple therapy (18%), other combinations (10%)
Clinical Study *Helicobacter pylori* Stool Antigen (HPSA) and PCR Assay Agreement

<table>
<thead>
<tr>
<th>Test Results</th>
<th>Total Cases</th>
<th>Initial Diagnosis Group</th>
<th>Test of Eradication Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=223)</td>
<td>(n=169)</td>
<td>(n=54)</td>
</tr>
<tr>
<td>PCR +</td>
<td>HPSA +/</td>
<td>PCR +</td>
<td>HPSA +/</td>
</tr>
<tr>
<td></td>
<td>PCR -</td>
<td>PCR -</td>
<td>PCR -</td>
</tr>
<tr>
<td>Number of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cases (%)</td>
<td>207 (92.8%)</td>
<td>16 (7.2%)</td>
<td>154 (91.1%)</td>
</tr>
<tr>
<td></td>
<td>15 (8.9%)</td>
<td>53 (98.1%)</td>
<td>1 (1.9%)</td>
</tr>
</tbody>
</table>

- Clarithromycin resistance-associated SNPs 81/207 (39%) positive samples
- Initial diagnosis test group, clarithromycin resistance-associated SNPs 47/154 (31%) positive samples

**Clinical Study Test of Eradication Group Treatment Regimens and *Helicobacter pylori* PCR Assay Results**

<table>
<thead>
<tr>
<th>Clarithromycin Resistance Detected by PCR</th>
<th>Total Cases*</th>
<th>Clarithromycin-based Triple Therapy**</th>
<th>Other Combination Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=52)</td>
<td>(n=89)</td>
<td>(n=23)</td>
</tr>
<tr>
<td>PCR +/resistance detected</td>
<td>Failure (n=29)</td>
<td>Success (n=63)</td>
<td>Failure (n=26)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>PCR +/resistance not detected</td>
<td>15</td>
<td>43</td>
<td>13</td>
</tr>
<tr>
<td>PCR negative</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>

*Success rate 13/27 (48%) when resistance predicted by PCR versus 43/58 (74%) when no resistance predicted (p=0.02)

**Success rate 9/22 (41%) when resistance predicted by PCR versus 30/43 (70%) when no resistance predicted (p=0.03)
Clinical Study Test of Eradication Group Treatment Regimens and *Helicobacter pylori* PCR Assay Results

<table>
<thead>
<tr>
<th>Clarithromycin Resistance Detected by PCR</th>
<th>Total Cases (n=54)</th>
<th>Previously Treated with Clarithromycin-based Triple Therapy (n=35)</th>
<th>Previously Treated with Other Combination Therapy (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR +/resistance detected</td>
<td>34 (64%)</td>
<td>24 (69%)</td>
<td>10 (56%)</td>
</tr>
<tr>
<td>PCR +/resistance not detected</td>
<td>19 (36%)</td>
<td>11 (31%)</td>
<td>8 (44%)</td>
</tr>
<tr>
<td>PCR negative</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

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Helicobacter pylori Diagnostic Algorithm

1. Mayo Clinic Laboratories utilizes the iFOCUS In-House Spectroscopy Test for the LineSa G Ensemble. Test performance characteristics for this algorithm have not been established for persons under age 6. For patients 6 to 17 years, age, weight, and height must be included in test results for appropriate result interpretation.

2. Serology is not recommended for patients with an active upper gastrointestinal bleeding or upper gastrointestinal symptoms, or patients with a known history of gastritis, peptic ulcer disease, or other upper gastrointestinal symptoms.

3. Pathogenic Helicobacter pylori infection should be considered if resistance to clarithromycin, metronidazole, or both is detected in a Helicobacter pylori Diagnostic Test.

4. Patients with previous eradication failure and non-compliance with prior eradication therapy should be retreated with a new therapy regimen.

5. Patients with a history of peptic ulcers or other upper gastrointestinal disorders should be considered for eradication therapy.

6. Patients with a history of peptic ulcers or other upper gastrointestinal disorders should be considered for eradication therapy.

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References


THANK YOU