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Alimentary Tract

# Helicobacter infections with rare bacteria or minimal gastritis: Expecting the unexpected

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ABSTRACT

*Background:* The routine use of special stains for detection of *Helicobacter* remains controversial.
*Aims:* To determine the frequency of histologically atypical *Helicobacter* infection.
*Methods:* All gastric biopsies received at a large pathology reference laboratory over a 6-month period were stained for *Helicobacter*, and the histologic and clinicopathologic parameters evaluated.
*Results:* Amongst 7663 *Helicobacter*-positive biopsies, 823 (10.7%) did not show typical chronic active gastritis with numerous *Helicobacter* organisms, and were therefore considered histologically atypical. Rare *Helicobacter pylori* organisms accounted for 58.0% of all atypical infections; the next most common atypical *Helicobacter* infection was that with minimal or no gastric inflammation (23.3% of atypical infections). Patients in these groups did not differ demographically from those with other forms of atypical or typical *Helicobacter* infection, although a small subgroup (6%) was more likely to have had a previously treated infection.
*Conclusions:* In many of these atypical infections, *Helicobacter* would not have been suspected based on the histologic findings alone, and would have been missed without routine special stains. Performing a sensitive stain could prevent additional testing and allow prompt treatment of the affected patients, thus substantially reducing the risk for peptic ulcer and gastric cancer and preventing the transmission of the infection to family members.


## 1. Introduction

*Helicobacter pylori*, rediscovered and conclusively identified in 1984 [1], was quickly recognized as the main cause of chronic gastritis and peptic ulcer disease [2], as well as a crucial risk factor for gastric cancer [3] and primary gastric B cell lymphoma [4]. Although *H. pylori* infection involves the entire stomach, both the intensity of inflammation and the bacterial load tend to be more intense in the gastric antrum, where the characteristic histologic pattern of chronic active gastritis is most readily recognized [5]. However, the spectrum of pathologic abnormalities associated with *H. pylori* infection has changed over the past decade, due at least in part to the widespread use of proton pump inhibitors (PPIs) in patients with gastroesophageal reflux disease and dyspepsia. In addition to their potent acid-suppression properties, PPIs also have substantial anti-inflammatory activity [6]. Furthermore, chronic use of these medications causes a decrease in the *H. pylori* population and a proximal shift of both bacteria and inflammation from the gastric antrum to the corpus [7–10]. As a consequence of these changes, fewer gastric biopsy specimens show typical *H. pylori* gastritis today than they did three decades ago.

The issue of the role of special stains in the detection of *H. pylori* has been debated for years, albeit with waxing and waning intensity. A spectrum of views has been proffered by pathologists, ranging from the claim that haematoxylin and eosin (H&E) staining alone is adequate to detect the bacteria, to the idea that immunohistochemical staining methods must be routinely used on all gastric biopsies regardless of the histology [11]. Many authors suggest that special stains should be performed when there is histologic evidence of gastritis but organisms are not visible on H&E stained sections [12–16]. This suggestion is based upon several studies and statements in textbooks, which assert that *H. pylori* infection is “always” associated with histologic evidence of significant gastritis [13,14,17]. However, the authors’ experience in evaluating gastric biopsies using special stains includes many examples of *Helicobacter* infection occurring in the absence of gastritis. In one recent editorial, Yantiss and Lamps stress the need

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for studies examining the value of special stains in gastric biopsies with minimal or no inflammation [15]. The studies performed to date have included only small numbers of *H. pylori*-positive biopsies, making it unlikely that less common histologic patterns of *H. pylori* infection would be encountered. As a result, we undertook the current study to determine the frequency with which, in a large population of patients, predominantly from the United States, *H. pylori* infection occurs in the setting of minimal or no gastritis.

## 2. Methods

### 2.1. Study setting

The study was conducted at the Gastrointestinal Division of Miraca Life Sciences, a specialized referral pathology laboratory that receives biopsy specimens from private outpatient endoscopy centres throughout the United States. The laboratory includes a group of 35 gastrointestinal pathologists who share a common approach to biopsy evaluation. Uniformity among pathologists is maximized through a standardized approach to specimen handling and a pre-determined set of diagnostic criteria and terminology for biopsy reading. The results of all surgical pathology cases are stored in a single searchable electronic SQL® database. In addition to histopathologic findings, the database contains the patients' demographic characteristics, clinical diagnoses, and either a summary or a complete report of the endoscopic procedure.

The Miraca Life Sciences Institutional Review Board determined that this study was entirely performed by analyzing existing data, documents, and reports, and that the information was recorded in such a manner that subjects cannot be identified. Therefore, pursuant to 45 CFR 46, section 101 b (4), the study was exempt from 45 CFR 46 regulations and no informed consent was necessary.

### 2.2. Gastric biopsy processing

All biopsy specimens designated as "stomach," "cardia," "corpus," "body," "fundus," "incisura," or "antrum" processed at the Miraca Life Sciences laboratories are routinely stained with H&E as well as with an anti-*Helicobacter* immunohistochemical stain (Hp-IHC) (Cell Marque, Rocklin, CA, USA). Slides processed in outside laboratories (approximately 10% of total gastric biopsies) are stained with either the Hp-IHC or a special stain (Hp Blue, Anatech, Ltd., Battle Creek, MI, USA) or Hp Yellow (DAKO, Carpinteria, CA, USA) for the detection of *H. pylori*. Special-stained or Hp-IHC-stained and H&E-stained slides are delivered to the pathologist simultaneously.

### 2.3. Study design and implementation

In the planning phase of the study, the authors held several consensus meetings to determine ways to categorize cases of *H. pylori* gastritis according to the difficulty of detecting organisms. Two categories, designated as "typical" and "atypical," were created. "Typical" cases were those demonstrating the characteristic chronic active gastritis with abundant *H. pylori* organisms (Fig. 1A and B). Histologically "atypical" cases of *H. pylori* infection were categorized as follows: 1. *Minimal or No Gastritis:* The histopathologic features of the gastric mucosa provided a low likelihood that *H. pylori* organisms would be present (Fig. 1C). Specifically, chronic inflammation was either absent or mild, although there could be changes reminiscent of reactive gastropathy such as foveolar hyperplasia. 2. *Rare Organisms:* Only extremely rare organisms (10 or fewer per histologic section) were detected by special staining (Fig. 1D). In many cases of this type in which HP Blue or HP Yellow stains were performed initially, a subsequent Hp-IHC stain was done because the histologic findings (i.e., the pattern of gastritis or the epithelial injury) were suspicious for *H. pylori* infection, but organisms were not identified on the initial stains. 3. *Oxyntic Gland Only:* Only oxyntic mucosa was present, and *H. pylori* stains revealed organisms confined to the deeper portions of the oxyntic glands or within the canaliculi of parietal cells (Fig. 1E). By definition, organisms were absent from the mucosal surface. 4. *Body Only*: Antral biopsies were negative for organisms, but biopsies from the body of the stomach were positive. 5. *H. pylori Located in an Unusual Site*: Organisms were present in an unusual location such as in the duodenum, in an esophageal inlet patch or in areas of intestinal metaplasia.

Cases with multiple gastric biopsy specimens, in which one or more was categorized as "atypical" for any of the reasons above, but at least one specimen was "typical" in appearance, were also noted, but were included in the typical category. When biopsies had histologic findings that fulfilled more than one atypical category, both categories were recorded, and the biopsy was assigned to the category corresponding to the atypical histologic feature that was more marked. After the two categories were defined, pathologists were instructed to assign all *H. pylori*-positive cases to either the "typical" or "atypical" category using a code that could be retrieved from the database. Both typical and atypical cases were coded so that failure to code a case would not result in an incorrect categorization.

At the end of the 6-month study period, the pathology reports of cases containing gastric biopsies, including all *H. pylori*-positive cases, were retrieved from the database using the previously entered diagnostic codes. The slides of all *H. pylori*-positive biopsies designated as "atypical" were reviewed by the authors. In addition, *H. pylori*-positive cases that had inadvertently not been categorized were examined and categorized appropriately; cases that remained unclassified were excluded from the analysis. The overall composition of the study groups is summarized in Fig. 2.

Biopsy specimens with atypical *H. pylori* gastritis were evaluated for a number of histologic parameters, including: site, number, and location of *H. pylori* organisms; degree of chronic inflammation and active inflammation (mild, moderate, or marked, following the criteria of the updated Sydney System [10]), and presence of lymphoid aggregates. Some atypical cases lacking full histologic data were not included in the detailed histologic analysis. In addition, a consecutive set of "typical" *H. pylori*-positive biopsies were evaluated for the same histologic features, for comparison with the atypical biopsies.

Clinical and demographic data (age, gender, indications for endoscopy), and endoscopic findings, including the sites from which the gastric biopsy specimens originated, were collected from the pathology requisition forms and endoscopy reports supplied by the clinician at the time of the procedure. Pathologic diagnoses for the gastric biopsies (in addition to *H. pylori* status) were extracted from the diagnostic codes entered at the time of diagnosis.

### 2.4. Statistical analysis

After all results were finalized they were entered into an Excel® database. The statistical analysis was carried out using publicly available online calculators (www.statpages.org). The analysis was focused on the relative prevalence of typical and atypical *H. pylori* infection in gastric biopsy specimens, and the association between atypical infection and various clinical and histologic features. Unadjusted odds ratios and their 95% confidence intervals were calculated to describe the strengths of the associations.

## 3. Results

### 3.1. Characteristics of the study group

During the 6-month study period, gastric biopsy specimens from a total of 82,709 endoscopies were obtained from 81,648 unique