

# Role of Culture in Cases of Perforated Peptic Ulcers Due To *Helicobacter Pylori*

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## Abstract

**Background:** The role of *Helicobacter pylori* infection in uncomplicated peptic ulcer disease has been definitively established through various studies since its identification. However, its association with perforated peptic ulceration is uncertain.

**Purpose:** This study was undertaken to establish the association of *H. pylori* in patients with perforated peptic ulcers and evaluate the role of culture for confirmation.

**Materials and Methods:** Intra-operative biopsy specimens from the site of peptic ulcer perforation, which tested positive by rapid urease test (RUT) in patients presenting to the hospital during the period of the study were aseptically collected and transported to the laboratory in Stuart's transport medium. The specimen was homogenized and primary smears were made for Gram-staining and plating done on Blood agar and Modified Thayer Martin agar. Incubation was done in a McIntosh Fildes' jar under micro-aerophilic environment for 7 days and any growth was identified using standard biochemical tests.

**Results:** Curved, Gram-negative bacilli morphologically resembling *H. pylori* were seen in 41.86% (18/43) specimens. Culture positivity of *H. pylori* was 18.60% (08/43).

**Conclusion:** Although the role of *H. pylori* infection in complicated cases seems to be less significant than in the causation of uncomplicated peptic ulcer disease, there is some degree of association between the two as evidenced by *H. pylori* positivity on RUT, Gram-staining and culture. However, this relationship between infection and perforation can be established upon undertaking further studies.

**Key words:** *Helicobacter pylori*, Perforated peptic ulcer, Modified Thayer Martin Medium

## INTRODUCTION

*Helicobacter pylori* is a small, Gram-negative, curved bacillus with a predilection for infecting the gastric mucosa. It is one of the most common bacterial pathogens of human beings and is found in the gut of half the population of the world. However, most of the infected persons are asymptomatic, only <30% are symptomatic.<sup>1</sup> Its

prevalence is highly variable in relation to geography, ethnicity, age, and socioeconomic factors, being high in developing countries and lower in the developed world. In developing countries, *H. pylori* infection is a public-health issue as it is markedly more prevalent at younger ages than in developed countries.<sup>2</sup> Annual incidence of *H. pylori* infection is 0.3-0.7% in developed countries as opposed to a much higher rate of 6-14% in developing countries.<sup>1</sup> *H. pylori* is implicated in the causation of many gastro-duodenal diseases including gastric and duodenal ulcers, active chronic gastritis and gastric cancer.<sup>3</sup> The ability of the organism to survive in the stomach despite the acidic pH can be attributed to the production of a strong, urease enzyme, which produces an alkaline microenvironment around the bacterium.

### Access this article online



www.ijss-sn.com

**Month of Submission :** 03-2015

**Month of Peer Review :** 04-2015

**Month of Acceptance :** 05-2015

**Month of Publishing :** 06-2015

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Although the role of *H. pylori* in the causation of uncomplicated, peptic ulceration is well established, the same does not hold true for perforated ulcer disease. Some studies have demonstrated a close relationship between ulcer perforation and *H. pylori* infection on histopathological examination. Demonstration of higher density of *H. pylori* in cases of perforated peptic ulcers indicates an underlying etiological connection.<sup>4</sup> Moreover, the presence of the organism in the mucosa and ulcer walls and a positive urea breath test in patients of acute ulcer perforation also indicates a significant role in the causation of the disease.<sup>5</sup> Culture is the gold standard for identification of *H. pylori*. However, the fastidious nature of the organism precludes the use of this method to demonstrate it in biopsy specimens. In this study, we aimed to identify the role of *H. pylori* in perforated peptic ulcer disease and evaluated culture as a modality for identification of *H. pylori* in biopsy specimens from such cases.

## MATERIALS AND METHODS

### Study Design

Place of study - Department of Microbiology, Lokmanya Tilak Municipal Medical College and General Hospital, Mumbai.

Duration of study: 1 year

Sampling method: Convenient sampling

All consenting patients presenting to the hospital with perforated peptic ulcer during the study period were included in this study.

### Methodology

Intra-operative biopsy specimens from cases of perforated peptic ulceration, which tested positive by rapid urease test (RUT) were taken and sent to the laboratory for identification of *H. pylori*.

### Sample Collection

Edge biopsy from the perforation site was collected after thorough toilet using sterile normal saline. The sample was put in Stuart's transport medium and transferred to the laboratory for processing within 30 min.

### Microbiological Processing

The biopsy specimen was homogenized by grinding it in a ground glass grinder and divided into two parts - one for Gram-staining and one for culture.

For Gram-staining, the biopsy sample was taken on a clean slide over an area of 2 cm<sup>2</sup> × 1 cm<sup>2</sup> with one drop of sterile normal saline. Gram-staining was done using freshly prepared Gram's reagents (Gram's crystal violet

and Safranin 0.5% w/v, Hi Media Labs Pvt. Ltd., Mumbai, India). Gram-negative, pale staining, short, plump, curved bacilli were considered to be suggestive of *H. pylori*<sup>6</sup> (Figure 1).

For culture, the homogenized biopsy specimen was streaked on freshly prepared blood agar and Modified Thayer-Martin agar (Thayer Martin Hi Veg Medium Base MV, Hi Media Labs Pvt. Ltd., Mumbai, India) augmented with 7% sterile, lysed blood and VCN supplement (Vancomycin, Colistin and Nystatin, Hi Media Labs Pvt. Ltd., Mumbai, India) to inhibit the growth of contaminant gut flora.<sup>7</sup> The plates were incubated in a McIntosh Fildes' anaerobic jar under micro-aerophilic condition for 7 days. The plates were examined for growth every 48 h. Growth generally appeared by Day 5.<sup>6</sup> If no growth of *H. pylori* appeared by Day 10, plates were discarded, and the specimen labeled as negative for *H. pylori*. *H. pylori* isolates were identified by typical colony morphology. Small, gray, translucent colonies were seen on Modified Thayer Martin Medium<sup>6,8</sup> (Figure 2) and blood agar. Biochemical identification was done by a positive catalase test, oxidase test and urease test and the inability of the isolate to hydrolyze hippurate and reduce nitrates to nitrites. Resistance to nalidixic acid was also seen.<sup>6,8</sup>

## RESULTS

Out of the forty-three intra-operative biopsy samples received, 62.79% (27/43) were from duodenal perforations and 37.21% (16/43) were from gastric perforations. Of these, 41.86% (18/43) showed bacilli morphologically resembling *H. pylori* on primary smear and 18.60% (08/43) were culture positive. Duodenal perforations showed more positivity with 48.15% (13/27) on direct staining and

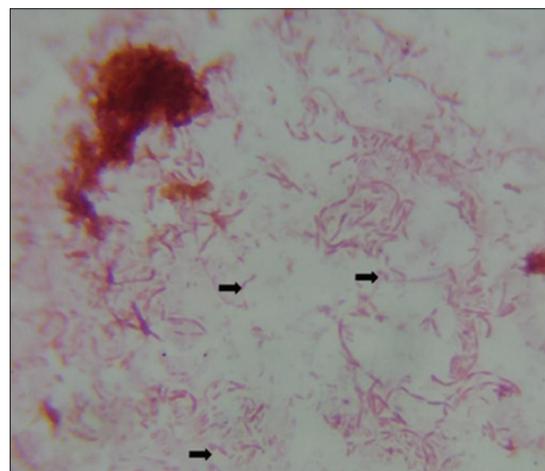


Figure 1: Gram-stain showing pale staining, Gram-negative, curved bacilli morphologically resembling *Helicobacter pylori* (Magnification ×1000)

25.93% (07/27) by culture. Gastric perforations showed presence of *H. pylori* in 31.25% (05/16) specimens on direct staining and 6.25% (01/16) on culture (Table 1).

Eight isolates were recovered on Modified Thayer Martin medium yielding a culture positivity of 18.60%. Blood agar grew five isolates with a positivity of 11.62%.

## DISCUSSION

*H. pylori*, as a fastidious organism requires an enriched medium with the appropriate micro-aerophilic environment to grow. Culture has been considered the gold standard in confirmation of the diagnosis of *H. pylori* infection in an individual. Although there are many non-invasive tests available for the rapid detection of the organism, like 13C urea breath test, 14C urea breath test, rapid urease detection test, stool antigen detection test etc., these tests provide only a provisional diagnosis of infection and culture is needed for confirmation.<sup>9</sup>

Very few studies have been carried out to demonstrate the role of *H. pylori* in the causation of peptic ulcer perforation. In this study, a total of fifty biopsy specimens were processed for the detection of *H. pylori*. Total specimens from duodenal perforations (62.79%) were more than gastric perforations (37.21%). *H. pylori* infects the mucus-

secreting epithelial cells of the stomach. Hence, infection and ulceration of the duodenum occurs only in cases of gastric metaplasia of the of duodenum.<sup>10</sup>

RUT is a screening test which can be used to predict *H. pylori* infection. In this study, all biopsies from perforated ulcers, which were positive on RUT were taken for further microbiological processing. Various methods have been employed for detection of *H. pylori* in biopsies from perforated ulcers like RUT, immuno-histochemical staining, histo-pathological examination (HPE) using hematoxylin-eosin stain or Giemsa stain and urea breath test.<sup>4,5,11</sup>

Mihmanli *et al.* showed the presence of the bacterium in mucosa and walls of perforated duodenal ulcers in 38.8% patients using hematoxylin-eosin staining.<sup>5</sup> Kumar *et al.* also reported *H. pylori* positivity on HPE of 33.72%.<sup>12</sup> Dogra *et al.* reported 42% biopsies from perforated ulcers as being positive for *H. pylori* on Giemsa staining.<sup>11</sup> These figures correlate well with the findings of this study where 41.86% of the ulcer perforations show bacteria morphologically resembling *H. pylori* on direct Gram-staining (Table 1). Culture for detection of the organism in perforated ulcers is not routinely done. Studies carried out by Chowdhary *et al.* in 1998<sup>13</sup> and Kumar *et al.* in 2004<sup>12</sup> used culture methods to identify the organism. However, in both studies, no *H. pylori* was isolated. Dogra *et al.* conducted a study in 2014 and reported a culture positivity of 20% from perforated ulcers.<sup>11</sup> This figure is similar to that seen in this study where 18.60% culture positivity for *Helicobacter pylori* was seen (Table 1).

Culture positivity in only 18.60% cases maybe due to the fastidious nature of the organism, presence of dead bacilli, overgrowth of contaminants from gut flora<sup>14</sup> or transformation of the bacteria from a cultivable, curved form to a non-cultivable coccoid form.<sup>15</sup>

Various factors such as biopsy site preparation, sample collection, transportation to the laboratory, delay in sample processing and the media used for isolation also influence the yield of the bacterium on culture.<sup>14</sup> These factors become even more important when potentially contaminated samples are to be processed, for example, in the present study, the sample was a biopsy specimen from perforated peptic ulceration. Such samples may be contaminated by the gut flora and primary isolation of *H. pylori* may be more difficult. Screening tests like RUT may be less specific in such cases due to the presence of other urease producing organisms such as *Klebsiella* spp., *Proteus* spp. and *Pseudomonas* spp. which may contaminate the perforation site. The use of selective, enriched media containing serum or lysed blood and antibiotics such as vancomycin, colistin, and nystatin helps to improve the



Figure 2: Small, gray, translucent colonies of *Helicobacter pylori* on Modified Thayer Martin Medium

**Table 1: Direct Gram-stain and culture positivity in biopsy specimens from duodenal and gastric perforations**

Total samples n=43		Direct Gram-stain positivity (41.86%)		Culture positivity (18.60%)	
Duodenal	Gastric	Duodenal	Gastric	Duodenal	Gastric
27	16	13 (48.15%)	05 (31.25%)	07 (25.93%)	01 (6.25%)

rate of isolation of the organism from such contaminated samples.<sup>14,16</sup> This may explain the higher efficacy of Modified Thayer Martin medium (18.60%) when compared to non-selective blood agar (11.62%) for the isolation of *H. pylori* in this study. Sang *et al.* also conducted a study where Modified Thayer Martin agar showed best results for the primary isolation of *H. pylori*.<sup>17</sup> However, Cuchi *et al.* reported that there is no significant difference in the isolation rates from Blood agar and Modified Thayer Martin agar.<sup>18</sup>

## CONCLUSION

Perforated peptic ulcers are a common cause of morbidity and mortality in the young population. *H. pylori* is implicated in a substantial number of these cases. While RUT maybe recommended as a screening test for *H. pylori* in uncomplicated ulcers, presence of urease producing contaminant flora in perforated ulcers reduces the specificity of the test in such cases. Therefore, Gram-staining and culture should be recommended for the diagnosis of *H. pylori* in cases of perforated peptic ulcers.

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**How to cite this article:** Tayal R, De A, Sundaravadanan S, Kumar M, Baveja S. Role of Culture in Cases of Perforated Peptic Ulcers Due To *Helicobacter Pylori*. Int J Sci Stud 2015;3(3):24-27.

**Source of Support:** Nil, **Conflict of Interest:** None declared.