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# DETECTION OF *HELICOBACTER PYLORI* FROM ENDOSCOPIC BIOPSIES AND THE BIOCHEMICAL CHARACTERISTICS OF THESE ISOLATES

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*Summary*

*Helicobacter pylori* (formerly *Campylobacter pylori)* has been recently described as a gastritis-associated bacterium. We examined endoscopic biopsies of 100 patients with dyspepsia and found H. *pylori* in the gastric antrum of 34 (34%) by either culture, urease tests and/or histology. Thirty-one out of 41 patients (75.6%) confirmed to have chronic active gastritis histologically had *H. pylori* in their gastric antrum compared to 3 out of 59 patients (5.1%) with dyspepsia but normal histology ($p<0.01$). Histological examination, using gram stain and the Warthin-Starry Silver stain, detected 29 of the 34 positive cases (85.3%); urease test, 26 cases (76.5%) and culture, 22 cases (64.7%). A combination of histological examination and urease test increased the detection rate to 97.1%. Therefore we felt that for the detection of H. *pylori* in endoscopic biopsies, culture, which is time consuming and expensive, is not necessary in routine diagnosis as it did not improve the diagnostic rate over a combination of histology and urease test. A comparative study on three media (blood agar, chocolate agar and Skirrow's agar) used in the isolation of .the organism showed that non-selective blood agar and chocolate agar were superior to Skirrow's agar. The strains isolated appeared to be homogeneous in their morphological and biochemical characteristics.

Keywords: Helicobacter pylori, chronic gastritis, urease test.

## INTRODUCTION

Recent work on upper gastrointestinal diseases have centred on *Helicobacter pylori* (previously *Campylobacter pylori).* This spiral bacteria had been observed in the stomachs of humans and animals several decades ago but had escaped isolation because of its microaerophilic nature.[1] It was not until 1982 that this organism was successfully cultured by Marshall and Warren who reported the association of H. *pylon* with chronic non-autoimmune gastritis.[2] Since then researchers throughout the world have corroborated their findings[3,4,5]

We describe here our experiences in the detection of H. *pylori* from endoscopic biopsies. Three types of media for its isolation were also compared and the biochemical characteristics of these local isolates presented.

## MATERIALS AND METHODS

100 patients with dyspepsia who attended the gastroenterology unit of the General Hospital, Kuala Lumpur were studied between April and December 1988. The first 4 patients on each endoscopy day were chosen for the study. Oesophagogastro-duodenoscopy was performed on each patient using an Olympus upper GI endoscope after an overnight fast. 5 gastric biopsies were taken about 2 cm from the pylorus using the Olympus FB 25K biopsy forceps. These biopsies were taken from an area of intact mucosa at a distance from any focal lesion, such as an ulcer or erosion. In between patients, the endoscope and biopsy forceps were thoroughly cleansed and soaked in 10% aqueous chlorlexidine solution for at least 5 minutes and then rinsed with clean tap water.

Two of the 5 biopsies were fixed in 10% buffered formalin and sent to the Histopathology department of the Institute for Medical Research (IMR) for histological examination using H & E staining and the Gram and Warthin Starry Silver stain for the organism. The classification of gastritis was based on Whitehead's Classification, with chronic active gastritis indicating flattened epithelium with decreased mucinogenesis, collections of polymorphs in gastric pits, diffuse inflammatory infiltrate of plasma cells, lymphocytes and polymorphs throughout the mucosa, some gland tubular reduction and occasional intestinal metaplasia.[6] Another 2 biopsies were placed at the side of a bijou bottle containing a small amount of normal saline. This was transported to the Bacteriology department at the IMR within 2 hours of collection. The saline ensured a humid

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environment but the tissues were not immersed in it as this would cause dissolution of the mucus layer and subsequent loss of bacteria into the saline.'

***Urease test:*** The last piece of biopsy was put into a 2% Christensen's urea broth for the urease test. A test was considered positive when there was a colour change from yellow to pink within 6 hours at room temperature.

***Isolation procedures:*** Blood agar (nutrient agar supplemented with 10% ox blood), chocolate agar (nutrient agar with 10% chocolated blood) and Skirrow's selective agar (Columbia agar base with 7% lysed ox blood, vancomycin 10 mg/l, polymixin B 1250 IU/l) were used.

The tissues were homogenised in tryptic soya broth using a Griffith's grinder, inoculated on each agar plate and incubated in a micro-aerophilic environment, comprising 5% 02, 10% CO2 and 85% $N_2$ for 5–7 days at 37°C. This environment was achieved by using an anaerobic jar containing a niicroaerophilic gas pak (Oxoid). Growth on the agar plate was semiquantitated 1+, 2+, 3+ according to its appearance on the primary inoculum, second and third streak.

***Morphological study:*** Gram stain and flagella stains were performed on all strains isolated.

***Biochemical tests:*** Cytochrome oxidase and catalase activity were tested according to the method of Cowen and Steel.[9] To detect nitrate reductase, bacteria grown on blood agar were heavily suspended in 1 ml of nutrient broth containing 0.1% $KNO_3$. After incubating at 37°C for 2 hours, nitrite reagents, sulfanilic acid and alpha-naphthylamine were added. Zinc dust was then added if the nitrates were not reduced. For nitrite reduction, the culture was heavily suspended in nutrient broth containing 0.001% $NaNO_2$, and the same reagents added after incubation at 37°C for 2 hours. H2S production was determined by lead acetate paper suspended over triple sugar iron agar which was then incubated in a microaerophilic environment for 3 days. Urease activity was tested in 2% Christensen's urea broth. Hippurate hydrolysis was tested with 0.4 ml. of 1% sodium hippurate solution in phosphate-buffered saline, pH 7.2. A heavy suspension was made and, after incubation at 37°C for 2 hours, 0.2 ml of ninhydrin was added. The development, within 10 minutes, of a deep blue colour was considered a positive reaction.'[0] Susceptibility to nalidixic acid and cephalothin was tested with the use of 30 ug discs. A loopful of the organism was inoculated directly onto blood agar plate. Reading was made after 3 days of incubation in a microaerophilic environment at 37°C. The organism was considered resistant if it grew right to the edges of the disc.

## RESULTS

The ages of the patients studied ranged from 16 to 76 years with an average of 42 years. Of the 100 patients studied, 34 (34.0%) had H. *pylori* in their gastric antrum, demonstrated either by culture, urease test or histology. 31 out of 41 patients (75.6%) confirmed to have chronic active gastritis histologically had H. ***pylori*** in their gastric antrum compared to 3 out of 59 patients (5.1%) with dyspepsia but normal histology. This difference was statistically significant at $p < 0.01$.

***Detection of H. pylori by histological examination, biopsy urease test and culture:*** Table 1 shows the detection of H. ***pylori*** by the 3 methods. Histology using gram stain and the Warthin Starry Silver stain detected 29 out of 34 positive cases (85.3%), urease test 26 cases (76.5%) and culture 22 (64.7%). The differences in detection rates possibly reflect the patchy distribution of the organism in the gastric mucosa or poor transportation of some of the specimens; 8 were received more than 2 hours after collection and 3 were immersed in normal saline at arrival in the laboratory. These might have affected survival and hence successful culture of the organism. A combination of histology and urease test or histology and culture increased the detection

**TABLE 1**
**DETECTION OF H. PYLORI BY HISTOLOGICAL EXAMINATION, BIOPSY UREASE TEST AND CULTURE**

| Method | Number positive |
|---|---|
| Histology + Biopsy urease test + culture | 16 |
| Histology + urease test | 6 |
| Histology + culture | 2 |
| Histology alone | 5 |
| Urease + culture | 3 |
| Urease alone | 1 |
| Culture alone | 1 |
| Total | 34 |

rate to 97.1% (33 out of 34 positive cases) in either combination.

***Comparison of culture media for the isolation of H. pylori:*** Chocolate agar and blood agar were better than Skirrow's agar for the isolation of H. ***pylori*** (Table 2). In 9 (40.9%) out of 22 instances, H. ***pylori*** was recovered from chocolate agar but not Skirrow's agar. The majority of growths were in 1+ quantity and appeared after 5 days' incubation on non-selective media. On Skirrow's agar, colonies took between 5 and 7 days to appear. As expected, a higher contamination rate was encountered in the non-selective media. 57% of the non-selective media were contaminated compared to 10% of the selective media. The majority of the contaminants were from the environment, ***Bacillus spp.*** being the most common.

***Morphology:*** The colonies were circular, convex, translucent, non-pigmented and about 0.5 mm in diameter. All the isolates were gram negative, slender curved or spiral rods. Flagella staining showed 3–4 polar flagella at one or both ends.

***Biochemical characteristics:*** Table 3 summarises the biochemical characteristics of the 22 local isolates. They have similar characteristics to the reference strain NCTC 11638.

## DISCUSSION

The discovery of Marshall and Warren opened up a wide area of investigation in gastrointestinal diseases. From our experience, H. ***pylori*** is not a difficult organism to culture, provided the conditions for transport and culture are strictly adhered to.

We have found that histological examination and the rapid biopsy urease test are a good combination for detecting the organism, obviating the need for culture which is time consuming and expensive. However, culture still has a specific role to play in the areas of pathogenicity and virulence as its aetiological significance in peptic ulceration is still in dispute. With the discovery of ***Gastrospirillum hominis,*** another spiral organism in the gastric mucosa, culture would also be useful to differentiate and define the roles of these organisms.[1] Furthermore, in the future, when the use of antibiotics is better established in the management of gastritis or peptic ulcers, susceptibility studies would require recovery of the organism.

We have found chocolate agar and blood agar to be superior to Skirrow's agar. Megrand ***et al.*** reported growth on non-selective Columbia agar with 5% sheep blood to be poorer and slower than on chocolate agar.[2] Some Japanese workers, however, reported better growth on Marshall's selective BHI blood agar and Skirrow's agar than on non-selective blood agar.[3]

Though contaminants were encountered more frequently on non-selective media, their presence did not affect the isolation of the organism.

The strains isolated appeared to be homogeneous in their morphological and biochemical characteristics.

TABLE 2
COMPARISON OF CULTURE MEDIA FOR THE ISOLATION OF H. PYLORI

| Quantity of growth | Chocolate agar | Blood agar | Skirrow's agar |
|---|---|---|---|
| 1+ | 17 | 13 | 12 |
| 2+ | 5 | 5 | 1 |
| 3+ | 0 | 1 | 0 |
| Total | 22 | 19 | 13 |

TABLE 3
BIOCHEMICAL CHARACTERISTICS OF H. PYLORI FROM ENDOSCOPIC BIOPSIES

| Biochemical characteristics | No. of strains n = 22 + | No. of strains n = 22 – | NCTC 11638 |
|---|---|---|---|
| Oxidase | 22 | 0 | + |
| Catalase | 22 | 0 | + |
| Urease | 22 | 0 | + |
| $H_2S$ (lead acetate) paper | 22 | 0 | + |
| Nitrite | 0 | 22 | – |
| Nitrate | 0 | 22 | – |
| Hippurate | 0 | 22 | – |
| Sensitivity to | | | |
| Nalidixic acid 30 ug | 0 | 22 | R |
| Cephalothin 30 ug | 22 | 0 | S |

R = resistant; S = sensitive

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