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Low Recovery Rate of Helicobacter Pylori from Positive CLO Test Patients Suffering from Dyspepsia

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Background: Helicobacter pylori are a well-known cause of gastrointestinal diseases particularly amongst patients suffering from dyspepsia.

Objective: To evaluate the recovery rate of Helicobacter pylori from suspected peptic ulcer patients with dyspepsia symptom.

Setting: Gastroenterology Unit, Aseer Central Hospital, Saudi Arabia.

Design: A Prospective Study.

Method: Gastroscopy and gastric biopsy were performed on 53 patients with dyspepsia from January 2012 to January 2013; all were subjected urease CLO test and culture. The CLO-positive biopsies were cultured using brain-heart infusion agar with added blood (7%), and Skirrow's supplement was used for isolating Helicobacter pylori. Inoculated plates were incubated at 37°C for 7–10 days in a microaerophilic incubation environment and examined for suspected Helicobacter pylori colonies. Helicobacter pylori cultures were confirmed by the positive urease, oxidase and rapid antigen test. Cultures of non-Helicobacter pylori bacteria were identified using few phenotypic tests then confirmed by VITEK 2 automated system.

Result: Seventeen (32.08%) Helicobacter pylori were isolated (in pure form or in mixed cultures) using Brain-Heart Infusion agar with blood and Skirrow's supplement. Nineteen (35.85%) samples revealed no growth, 5 (9.43%%) revealed the growth of Acinetobacter spp, 4 (7.55%) revealed Brucella melitensis, 2 (3.77%) revealed Pasteurella spp. and 1 (1.89%) revealed Pseudomonas aeruginosa.

Conclusion: The recovery rate of Helicobacter pylori from CLO positive biopsies was low, 17 (32.08%), but growth of other gram negative bacilli was documented.

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Dyspepsia is a common problem frequently caused by Helicobacter pylori (HP) associated gastritis and peptic ulcer[1]. HP is considered an etiological agent of peptic ulcer disease and mucosa associated lymphoid tissue (MALT) lymphomas and adenocarcinoma[2,3]. The prevalence of HP infection worldwide is approximately 50% and as high as 80%-90% in developing countries[4,5]. Approximately 20% of people infected with HP develop gastro-duodenal disorders during their lifetime. Low socioeconomic status, overcrowding, and poor sanitation are well-recognized risk factors[6]. Until now, no method has adequate sensitivity and specificity to be considered a gold standard for diagnosis of HP[7,8]. The innovative isolation of HP by Warren and Marshall in 1983 from peptic ulcer disease has changed the traditional perception of our understanding of gastroduodenal ulcer disorders[4,9]. Subsequent studies indicated a positive correlation between HP infection and the occurrence of peptic ulcers[2,10]. The prevalence of HP infection exhibits a substantial geographical variation. It is reported that 60% to 70% of patients with gastric ulcer, and 90% to 95% of patients with duodenal ulcer have marked gastric colonization of HP[11,12].

A patient or local community with high incidence of dyspepsia should be investigated for H. pylori infection. If the infection is confirmed, the disease could be alleviated by medication[6,13].

The aim of the study is to evaluate the recovery rate of Helicobacter pylori from suspected peptic ulcer disease patients with dyspepsia symptoms.

METHOD

Adult patients with dyspepsia or patients suffering from upper gastrointestinal bleeding were enrolled in the study from January 2012 to January 2013. Patients who had taken antibiotics or acid suppressive agents in the past four weeks, have chronic liver disease or cancer were excluded. Patients gave informed consent to participate in the study.

Upper GI endoscopy was performed in patients suspected of having peptic ulcer disease and after confirming the peptic ulcer disease biopsies were taken from the antrum of the stomach and used for both CLO test and culture. The CLO test was performed in the endoscopy room. A definite magenta color was required to read the test as positive.

Tissue biopsy was placed in 0.2 mL sterile saline and then placed in a sterile Petri dish and minced with two sterile scalpel blades. Specimens were inoculated onto brain-heart infusion agar plates with 7% blood plus Skirrow's supplement (trimethoprim, 5 mg/L; vancomycin, 10 mg/L; polymixin B, 2500 units/L).

Inoculated plates were incubated at 37°C for 7–10 days in a microaerophilic incubation environment and examined every other day. H. pylori colonies are typically small, flat and translucent to gray, see figure 1. Plates were examined until the tenth day before reporting a negative growth.

Isolates were tested for urease, oxidase and catalase production and examined microscopically after Gram stain. The presence of Helicobacter pylori was confirmed by the presence of gram-negative curved bacilli and a positive test for urease, oxidase and catalase production.

The VITEK 2 (Biomeraux) automated system was used for confirming non-H. pylori bacteria following protocols described by the manufacturer.

Helicobacter pylori antigen test was performed according to the manufacturer's instructions. A sample was considered positive when a purple-pink line (test line) revealed, in addition to the control line and was considered negative when only the control was shown. The results were read one time within 15-minutes of the incubation period and later after 30 and 60 min.

RESULT

H. pylori was isolated (in pure form or in mixed cultures) from 17 (32.08%) specimens. Colonies of H. pylori were small, flat and translucent to gray. Plates were examined until the tenth day before reporting a negative growth, see figure 1.

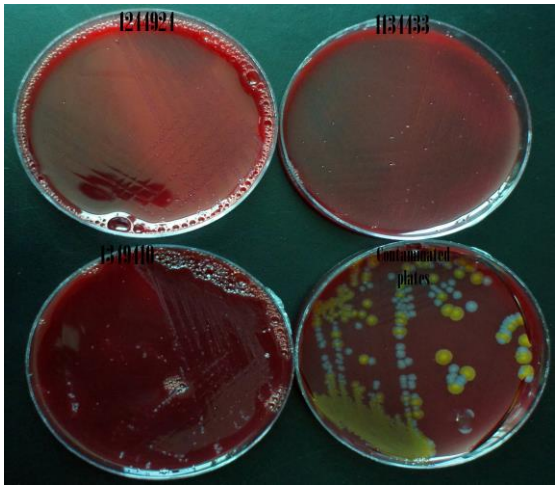

Figure 1: Growth of Helicobacter pylori after purification with subcultures showing small glistening translucent colonies (samples #1244921, #1134433, and #1319416), and a contaminated plate (bottom right) on Brain-Heart Infusion agar plates with 7% blood plus Skirrow's supplement after 7 days incubation at 37^{o}C under 10% CO_2 atmosphere

The presence of H. pylori was confirmed by the presence of gram-negative curved bacilli and a positive test for urease, oxidase and catalase production, see figure 2.

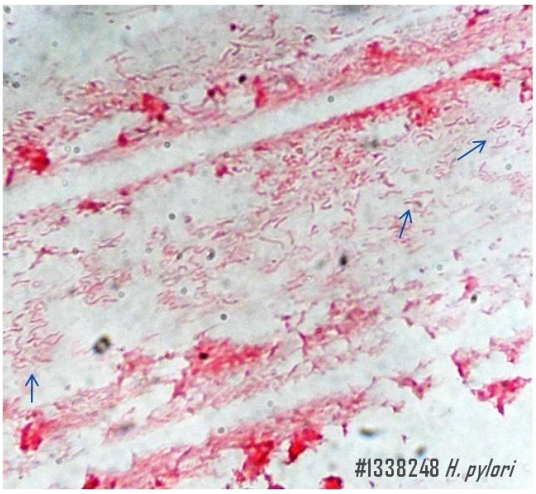

Figure 2: A Gram-Stained Slide Prepared from 7-Day-Old Culture of Helicobacter Pylori Recovered from a Patient with Dyspepsia (Sample #1338248). Note: The Gram-Negative Curved Bacilli

H. pylori was isolated (in pure form or in mixed cultures) from 17 (32.08%) specimens. Nineteen (35.85%) revealed no growth; five (9.43%) revealed the growth of Acinetobacter spp, four (7.55%) revealed the growth of Brucella melitensis, one (1.89%) had Pasteurella canis, one (1.89%) had Pasteurella pneumotropica, one (1.89%) had Pseudomonas aeruginosa 2 (3.77%) have been contaminated by moulds and three (5.66%) were unidentified gram negative bacteria, see figure 3.

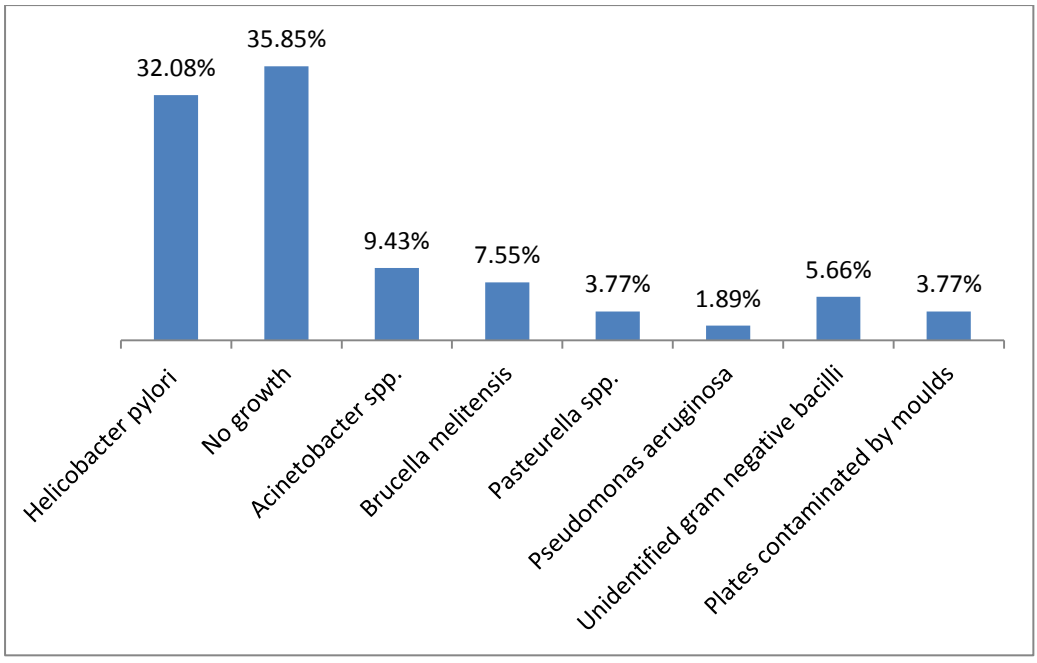

Figure 3: Analysis of Bacterial Isolates from Biopsy Specimens of Patients with Dyspepsia (N = 53) Processed for Culture Showing the Percentage of Helicobacter Pylori in Relation to Other Isolates

DISCUSSION

Helicobacter pylori (HP) eradication is the mainstay of treating patients with peptic ulcer disease. Therefore, the Helicobacter status of such patients should be identified using a reliable test. HP infection could be identified by endoscopy and biopsy. The specimen is subjected to histological examination, culture, and rapid urease (CLO) test. In addition, HP infection could be identified by serology, the urea breath test, or detection of H. pylori antigen in the stools. Urea breath test and the stool antigen test detect active infection only. However, no single test can be relied upon to detect definitely colonization by H. pylori, and a combination of two is recommended if this is feasible[14].

The bacteriological features of HP have gained enormous attention regarding methods of detection, identification and eradication[15]. HP are known to be difficult to grow on culture media[16]. The present study showed low isolation rate of Helicobacter pylori (32.1%) and the majority (35.8%) of the samples revealed no growth. The high negative result has been previously reported in patients with non-Helicobacter pylori gastritis[17]. Another study found that 45.9% of gastric ulcer and 29.6% of duodenal ulcer patients were Helicobacter pylori culture-negative[18]. Furthermore, Helicobacter pylori-positive culture was found to be dependent on the type of pathology involved[19]. Among patients with chronic inflammation of mucosa, positive Helicobacter pylori culture was found in 50% of the patients whereas the recovery rate was 19% among patients with gastric bile reflux; those authors suggested that the rapid urease (CLO) test was unreliable for exclusion of H. pylori infection during the acute phase of ulcer bleeding[19,20]. In another study, non-HP bacteria were isolated from 65% of gastric samples where the dominant isolates were Streptococcus, Neisseria, Rothia and Staphylococcus, which differed from the predominantly acid resistant species reported previously in healthy volunteers[21].

Another explanation for the low HP recovery rate in the present study is that there were a number of patients with non-ulcer dyspepsia where the occurrence of non-HP bacteria such as the urease producing bacteria staphylococcus epidermidis, staphylococcus warneri, staphylococcus capitis, staphylococcus aureus, brevibacterium species, and klebsiella pneumoniae was found significantly higher compared to patients with gastric ulcer[22,23].

In the present study, a significant number of non-HP bacteria were encountered, namely: Acinetobacter spp. 9.4%, Brucella melitensis 7.5% and Pasteurella canis 3.8% and Pseudomonas sp. 1.9%. These bacteria grew in spite of the presence of the Skirrow's selective antibiotic supplement. These non-HP bacteria may have a role in the pathogenesis of chronic inflammation of stomach mucosa, but this needs further confirmation[23]. Colonization and overgrowth of urease-producing bacteria other than HP have been found to induce false-positive urea breath test. These false-positive results have been induced by urease-producing Proteus mirabilis in patients treated with H2 receptor antagonists for long periods and by urease-positive Micrococcus species in the gastric mucosa. Streptococcus, Staphylococcus, Gardnerella, Lactococcus and Enterococcus have strong urease activity and were isolated from the stomachs of hypochlorhydric patients, they are responsible for false-positive results in the urea breath test. Yeast-like micro-organisms could give false-positive results in urea breath test[24,25].

It is known that the enzyme urease is produced by many taxonomically miscellaneous bacterial species, including normal flora. This fact could be behind the high number of CLO test-positive but culture-negative (35.8%) specimens. Thus, the effect of stomach bacteria ought to be taken into account when interpreting the results[14]. Worldwide, CLO test is one of the most widely used rapid urease test for the diagnosis of Helicobacter pylori infection but

its results should be interpreted with care. Moreover, false positive results could also occur with the urea breath test due to urease-producing bacteria as reported by Osaki et al[22]. Therefore, mouth washing prior to a standard UBT is recommended[26]. The reliability of the CLO test has been questioned, especially in patients with bleeding peptic ulcers. On the contrary, histology was found to be a fairly consistent test, despite the presence of bleeding[8,27].

CONCLUSION

The recovery rate of Helicobacter pylori from patients with dyspepsia with or without upper gastrointestinal bleeding or bleeding who had a positive CLO test was low. This could be due to urease producing non-Helicobacter pylori bacteria, bleeding ulcers, or due to non-ulcer dyspepsia without Helicobacter pylori. Such issues related to the CLO test positive-Helicobacter pylori and negative cultures situations amongst patients with gastrointestinal disease need further investigations.

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