ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Comparison of Rapid Urease Test and Culture in the Diagnosis of *Helicobacter pylori.*

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ABSTRACT

A total of 109 endoscopic biopsy samples were collected from alcoholic and non-alcoholic patients to detect the presence of H. pylori using conventional methods. 82.6% of the cases (n=109) were positive by Rapid urease test. 23.9% (n=109) of the cases were positive by culture. The low isolation rate may be due to the distribution of H. pylori in gastric mucosa, its fastidious nature, administration of antibiotics (for other infection) and PPI. The simple and inexpensive Rapid Urease Test and Giemsa stain detected the maximum number of positive cases.Culture for H. pylori was low, revealing that isolation of H. pylori by culture is possible in reference laboratories.

Keywords: Rapid urease test (RUT), *Helicobacter pylori*.

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ISSN: 0975-8585

INTRODUCTION

Several diagnostic modalities are available to detect *H.pylori* infection. The two major categories of diagnostic assays are endoscopic or invasive tests and non-endoscopic or noninvasive tests. The endoscopy based tests include rapid urease, histopathological evaluation, polymerase chain reaction and culture. The non-endoscopic tests include antibody detection methods and carbon labeled urea breath test. The choice of the tests depends on the laboratory resources and the clinical situation whether for diagnosing infection or for documenting eradication.

H.pylori is a robust producer of urease and its presence is detected by rapid urease tests. The advantage of these tests is that they can be readily performed in the endoscope suite. Another rapid test is smear evaluation of the specimen. Imprint smears stained by rapid Giemsa or Gram stain provide an adjuvant to histopathological examination of gastric biopsy specimens

Culture is probably the most difficult approach to the diagnosis of *H.pylori*. The advantages are that it is highly specific and the antibiotic sensitivity can be detected. High rate of false negatives due to the fastidious nature of the organism coupled with expense incurred for culture have limited its application

Chronic *H.pylori* infection elicits local and systemic immune response that lead to production of antibodies. The presence of IgG antibodies to *H.pylori* can be detected by immunoassays. Serology is sensitive for primary diagnosis but is not useful in assessing post treatment *H.pylori* status.

The urea breath test depends on the urease activity of *H.pylori* to detect the presence of infection. Sensitivity is excellent because the whole stomach is sampled. Unlike serology it is useful for determining the success of the eradication therapy. Even though the test is more accurate than serology its usage is limited due to high cost and lack of facilities for testing.

With the advent of PCR, many possibilities have emerged for diagnosing *H.pylori* infection. PCR allows identification of the organism in samples with few bacteria and it has been successfully used to detect *H.pylori* CagA and VacA virulence genes in gastric biopsy samples. The potential advantage of PCR includes high specificity, quick results and the ability to identify different strains of bacteria for pathogenic and epidemiologic studies. The major limitation of PCR is that relatively few laboratories currently have the capacity to run the assay. Cultural Characteristics and Growth Requirements

H. pylori are strictly micro-aerophilic, requiring C02 (5-20%) and high humidity for growth. H. pylori require media contain¬ing supplements such as blood, haemin, serum, starch or charcoal. Growth is best on media such as moist freshly prepared heated (chocolated) blood agar, or brain-heart infusion agar with 5% horse blood and 1% IsoVitaleX.

Strains grow in various liquid media supplemented with fetal calf or horse serum. Some strains grow in serum-free media, notably bisulphite-free brucella broth. All strains grow at 37°C, some grow poorly at 30°Cand 42°C but none grows at 25°C [1].

They are weakly hemolytic on 5% horse blood agar. Motility is weak or absent when grown on agar. H. Pylori are inactive in most conventional biochemical tests. Notable exceptions are the strong production of urease16, catalase and alkaline phosphatase. All strains produce DNAase, leucine aminopeptidase, and glutamylaminopeptidase [2].

Laboratory Diagnosis

The two major categories of diagnostic assays for *H.pylori* are endoscopic, or invasive, tests and non-endoscopic, or noninvasive test.

	Diagnostic test	Method of organism Identification
Invasive	Rapid urease test (RUT)	by urease production
	Histology	by morphologic features and location
	Culture	by biochemical properties
	Polymerase chain reaction (PCR)	by genetic sequencing
Non-invasive	Antibody detection	by immunologic response
	Urea breath test	by urease production

The stomach is usually accessed by fiber optic endoscopy, and biopsy specimens are obtained. Using two contrast stains, topical acriflavine and intravenous fluorescein, with a confocal laser endomicroscope, endoscopists were able to detect see clusters H. pylori on the surface and in the deeper layer of the gastric epithelium [3]. This technique enabled detection of by surface microscopy imaging of living tissue during ongoing endoscopy for the first time.

It is possible that gastric juice obtained by a nasogastric tube allows the detection of H. pylori by culture, staining, urease test, and PCR, but it is less reliable than gastric biopsy specimens. The string test can also be used to obtain gastric mucus [4] however, the most attractive method seems to be an extendable oro-gastric brush contained in a plastic tube. The brush is swallowed, extended into the stomach to brush the mucosa three or four times, retracted in the protective sleeve, and withdrawn from the patient. This method is rapid and appears to be reliable for H. pylori infection diagnosis [5].

Specimen Collection

The best specimens to culture H. pylori are biopsy samples obtained during endoscopy. The recommendation is not to consume Proton pump inhibitor for 2 weeks prior to endoscopy.

Transport of biopsy specimens

H. pylori are a fragile organism and must be protected from desiccation and contact with oxygen and room temperature. It is mandatory to place them either in a saline solution for short-term transport (4 h maximum) [6] or in a transport medium, usually consisting of semisolid agar, maintained at 4°C. A commercially available medium, Porta-germ pylori is effective for this purpose. Storage at 4°C in a medium containing 20% glycerol also led to H. pylori recovery in 81% of the biopsy specimens' tested [7].

Grinding of biopsy specimens

Comparison of culture performed with and without grinding showed a higher number of colonies after grinding, for this reason grinding of the biopsy specimen is mandatory [8].

Media

The media components include an agar base, growth supplements and selective supplements. Most agar bases are satisfactory for growing H. pylori, e.g., brain heart infusion agar, Columbia agar. Concerning the growth supplement, it is mandatory to add blood or serum, which includes numerous nutrients (vitamins and oligoelements, etc.) which enhance *H.pylori* growth.

The proportion of blood or serum can be 5%, 7%, or preferably, 10%. Red blood cells can be lysed for these growth substances to be more readily available. Animal blood, e.g., sheep or horse blood, can be added. Other growth supplements such as starch [9], bovine serum albumin [10] and Cyclodextrins, which are cyclic oligosaccharides produced from starch by enzymatic treatment retaining the same properties as starch, are employed [11].

Different selective supplements containing antimicrobial compounds have been proposed: vancomycin or teicoplanin to inhibit gram-positive cocci; polymyxin, nalidixic acid, colistin, trimethoprim, or cefsulodin to inhibit gram-negative rods; and nystatin or amphotericin B to inhibit fungi.

Non selective media such as Chocolate agar, Brain heart infusion agar with 5% horse blood, Brucella agar with 5% Sheep blood and Tryptone Soya agar with 5% sheep blood can be used. Selective media include Skirrows Campylobacter Medium and Brain heart infusion agar with vancomycin [6ug/ml] nalidixic acid [20ug/ml] and amphotericin [2ug/ml] have given good recovery.

Several studies performed in the early days of H. pylori detection showed the importance of using both a nonselective medium and a selective medium [12].

A critical point is to use fresh media (less than a week old) which is kept in closed boxes at 4°C to maintain humidity and avoid light exposure. Helicobacters are microaerophilic and capnophilic. Several systems can be used to achieve a microaerobic atmosphere, from the most sophisticated systems, such as a microaerobic cabinet or an incubator with an adjustable gas level, to jars in which the adequate atmosphere is created with an automatic apparatus or with H2-C02-generating packs.

The atmosphere in jars will vary according to the quantity of bacteria consuming oxygen; therefore, the gas pack should be changed every other day. While H. pylori growth is possible in a candle jar [13], it takes a longer time and results in small colonies.

The optimal culture temperature is 37°C, testifying to the adaptation of this bacterium to humans. For primary culture under optimal conditions, colonies may appear after 3 days and are at their optimum on day 4. However, in the case of negative culture, a 7- to 10-day incubation is recommended to ensure that the result is negative; if only a few organisms are present, this time lapse may be necessary to visualize the colonies.

In contrast, subcultures only take 2 to 3 days. When few colonies are present, the recommendation is to subculture by plating the colonies on a small area of the agar plate. It is important to remember that once *H. pylori* reaches its growth plateau, it becomes coccoidal and loses its viability, most likely due to a lack of adequate nutrients.

Broth culture

Brain heart infusion or Brucella Broth with 1-10% fetal calf serum[14] may be preferable for studies on physiology and metabolism.

Identification of *Helicobacter pylori* in culture [15]

The growth of small, circular, smooth grey and translucent colonies observed after 3 to 4 days on the selective media plated with gastric biopsy specimens is an important criterion for H. pylori identification.

Gram staining of the colonies reveals gram negative curved rods, the spiral forms being less obvious .The characteristic gull wing is seen in broth cultures. Motility is best demonstrated in broth cultures and is weak when grown on agar.

The identification of culture consists essentially of testing for the presence of certain enzymes: cytochrome oxidase, catalase, and urease which are positive.

The ApiCampy strip20 identification of H. pylori via positive urease, glutamyl transpeptidase, and alkaline phosphatase and negative nitrate reductase and hippuricase. Its resistance to nalidixic acid and sensitivity to the antibiotic cephalothin, helps in distinguishing it from other species.

Histopathological Diagnosis [16]

H.pylori can be identified with haemotoxylin and eosin but the bacteria can be more reliably seen with special stains such as acridine orange, modified Giemsa, cresyl violet or warthin-starry stains.

The typical morphology of *H.pylori* is a comma shaped bacillus observed on the epithelial surface.

Gram staining of the touch smear of the biopsy specimen by rubbing it forcefully on a glass slide was used to confirm the presence of Campylobacter pylori by Montgomery et al 1988 [17] this method had a sensitivity and specificity of 92% and 100% respectively.

Nijhawan et al [18] used the gastric crush cytology in the detection of *H.pylori* infection and highlighted the advantage of crush smears

Warthin - Starry silver stain demonstrates *H.pylori* clearly as spiraled black rods against a yellow background. In Giemsa - stained sections, the organisms are clearly visible as Giemsa - positive (dark blue) spiraled rods.

M. Anjana et al [19], evaluated the staining method of impression smear by Gram, two-step Gram, dilute carbol-fuchsin and Giemsa.

Urease Tests

The discovery that H. pylori are a strong urease producer was made by Langenberg et al [20] and was used for rapid diagnosis by McNulty and Wise [21]. When a biopsy specimen containing H. pylori is introduced into a urea-rich medium, the urease hydrolyses the urea down into carbon dioxide and ammonia. The ammonium ion increases the pH, and a pH indicator, e.g., phenol red, changes color, in this case from yellow to red.

Modifications include McNulty and Dent [21] buffered 40% urea solution and Hazell's [22] solution with a high concentration of urea and pH indicator .Standardization of urease test was studied by Vinci. S .Jones et al. The various factors such as the concentration of urea in broth need for buffering the solution and addition of antibiotics were investigated [23].

AV Thillainayagam et al described the use of an ultra-rapid endoscopy room test in which unbuffered urea solution with indicator was used. The test had a sensitivity and specificity of 89% and 100% [24]**.**

Commercial Kits

The first-generation commercial kits were agar based, e.g., the CLO test. The new generation kits introduced in 1995 are strip-based tests

In the first study, Rogge et al60, compared this new test to the CLO test which showed 99% sensitivity and 95% specificity after 2 h, which is superior to those of the CLO test.

Polymerase chain reaction [PCR] [25]

The PCR was developed in the 1980s and thereafter quickly applied to the detection of H. pylori. Its application in the field of H. pylori concerns not only the detection of the bacterium but also its quantification and detection of specific genes relevant to pathogenesis (CagA) and specific mutations associated with antimicrobial resistance.

The first targets used were the genes of the urease operon: ureA and glmM, or the 16S rRNA gene.

Two main pathogenic factors the Cag PAI and the polymorphism of the VacA gene and other genes involved in adherence (babA2, sabA) or in pathogenicity (oipA, dupA, iceA) can also be detected by PCR. The new real time PCR technique is considered a breakthrough as it allows quantification and detection of point mutation associated with antibiotic resistance.

Non- Invasive Test

The first method used was serology. However, due to the difficulty in obtaining an optimal specificity, other methods have been proposed namely Urea breath test, stool antigen test, and most recently, detection of specific antibodies in urine or saliva.

Urea Breath Test [UBT] [26]

A solution of labeled urea ingested by the patient is rapidly hydrolyzed by H. pylori urease, the labeled CO2 is absorbed by the blood and exhaled in expired air. If the patient is not infected, most of the isotope is eliminated in urine without modification.

When [13C] urea is used, a specimen collection is performed before and 30 min after the ingestion.

Aim

The aim of this study is to compare rapid urease test(RUT) and culture sensitivity in the diagnosis of *H.pylori* infection.

Objectives

1. To isolate and identify *H.pylori* by conventional methods.
2. To detect the prevalence of *H.pylori* in alcoholic and non-alcoholic gastritis patients.
3. To compare the prevalence of *H.pylori* in alcoholic and non-alcoholic gastritis patients.
4. To correlate the conventional laboratory test findings with endoscopic clinical diagnosis.stion.

MATERIAL AND METHODS

Ethical Consideration

The study was conducted with the approval from the institutional Ethical Committee, Sree Balaji Medical College and Hospital, Chennai-44. Permission to conduct the study was sought from the respective hospital authorities. Informed consent was obtained from the patients before the enrolment into the study.

Period of Study

This is a prospective cross sectional study conducted over a period of eighteen months from May 2012 – October 2013.

Place of Study

This study was carried out at The Department of Microbiology in collaboration with The Department of Surgical Gastroenterology, The Department of Medical Gastroenterology, The Department of Pathology Sree Balaji Medical College and Hospital, Chennai-44.

Study Group

Outpatients and inpatients, of both sexes in the age group 20 – 70 years, based on the following criteria were included in the study.

Inclusion criteria

- Patients with complaints suggestive of upper gastro intestinal diseases in Alcoholic and Non-Alcoholic Gastritis.
- Patients with antral gastritis, duodenitis, gastric ulcer and duodenal ulcer.
- Patients who were not on antibiotics, proton pump inhibitor or Helicobacter eradication therapy within 1 month prior to inclusion in this study.

Exclusion criteria

- Patients with previous gastric surgery.
- Patients with active bleeding.
- Gastric carcinoma.

Study Design

The details of complete history, clinical features of the patients to be subjected to endoscopy were obtained. Preinvasive procedure preparation for Oesophago-gastro-duodenoscopy was performed as per norms. Biopsy tissue was collected from the gastric antrum of the patient and the specimens were submitted to Rapid Urease Test, Gram stain, Giemsa stain, Culture and Sensitivity and Histopathological study. A patient with Helicobacter pylori infection was defined as those patients who were positive for at least two out of the evaluation tests.

Specimen Collection and Transport

Biopsy Sample

Patients fasted overnight before endoscopy. Endoscopy was done using fiber optic endoscope. The endoscope and the biopsy forceps were rinsed thoroughly with water and soaked in 2% gluteraldehyde for 20 minutes77 and were thoroughly rinsed with sterile normal saline just before the collection of specimen.

Four biopsy samples were taken from the antrum (2 cm from the pylorus) and were transferred to respective Eppendroff tube under sterile conditions. One sample was inoculated into urea broth for rapid urease, two specimens were transported in normal saline for culture, Gram stain and Giemsa stain and the last specimen was placed in 10% formalin for histopathological examinations.

The specimens for culture were transported to the laboratory and were inoculated on the culture media without delay.

Processing of Specimens

Rapid Urease Test

An antral biopsy tissue was placed in an Eppendorf tube containing 1 ml of Rapid urease test broth (HiMedia RUT broth M1828, prepared as per the manufacturer's instruction.). Colour change from yellow to pink at room temperature within two hours, were taken as positive [27].

Culture

Biopsy tissue was crushed between two sterile glass slides and the minced tissue was inoculated onto freshly prepared campylobacter agar base with 5% defibrinated sheep blood and Campylobacter Selective Supplement and chocolate agar (non selective media).The plates were incubated at 37°C in a candle jar with a pad of cotton soaked in water placed at the bottom. The plates were examined for bacterial growth between three to seven days. Characteristic small, translucent circular colonies were confirmed by gram stain, catalase, oxidase and urease. Antimicrobial sensitivity was performed by Disc diffusion method using commercially available antibiotic discs.

Confirmatory tests for suspected colonies

1. Gram stain-Gram negative curved bacilli were seen.
2. Oxidase test-The suspected colony was streaked on the surface of oxidase disc containing 1% tetramethyl paraphenylene diamine dihydrochloride. An intense purple colour developed within 5 seconds and was recorded as positive. Positive and negative controls were used.
3. Urease test - The colony was emulsified in 0.5 ml of the urea broth. An instant colour change from yellow to pink was noted as positive.
4. Catalase test - The suspected colony was introduced with a glass rod into 3% Hydrogen peroxide taken in a clean test tube. Immediate production of gas bubbles was noted as positive. Positive and negative controls were also tested.

Crush cytology

ISSN: 0975-8585

Another biopsy tissue was crushed between two sterile glass slides and the minced tissue was used to make to two smears.

Gram stain

One of the slides was air dried and heat fixed. The slide was covered with methyl violet for one minute, excess stain was poured off, Grams iodine was added and washed after 1 minute. This was followed by acetone for 2-3 seconds. The acetone was washed and the slide was counter stained with dilute carbol fuschin for one minute, washed with water, blotted dry and observed under oil immersion objective. Helicobacter pylori appeared as gram negative curved bacilli.

Giemsa stain

The other slide was air dried and fixed with methanol for 3 minutes, 2-3 drops of undiluted Giemsa stain was added and kept for 5 minutes. The smear was then washed with water, blotted dry and seen under oil immersion objective. The organism appeared deep purple with the typical gull- wing morphology.

Histopathology

One specimen was fixed in 10% formalin, paraffin sections were made and stained with Haematoxylin and Eosin and examined for Helicobacter pylori.

RESULTS

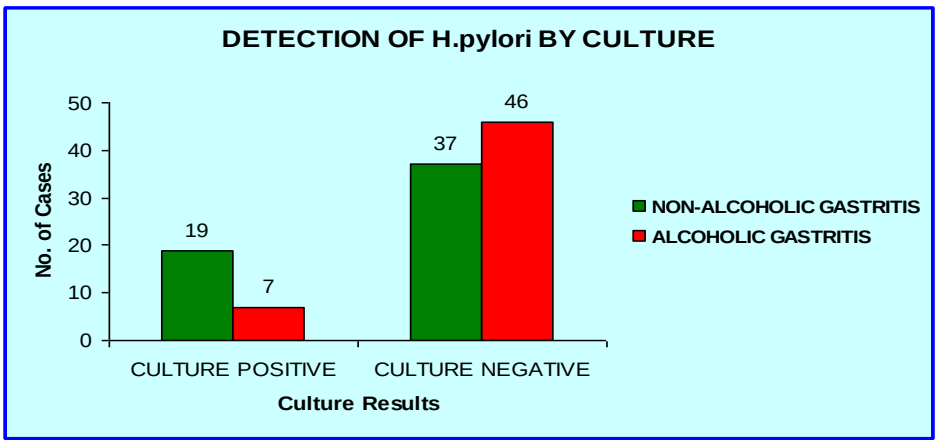
A total of 109 endoscopic biopsy samples were collected from alcoholic and non-alcoholic patients to detect the presence of H. pylori using conventional methods. The sample collected was processed immediately without any delay. RUT was done immediately in the endoscopic suite and the positive colour change was noted. Positive cases were further processed for staining, histopathological studies, culture and sensitivity.

Rapid Urease Positivity Vs Endoscopic Diagnosis

ENDOSCOPIC DIAGNOSIS	TOTAL	RAPID UREASE TEST POSITIVE
ANTRAL GASTRITIS	64	50
DUODENITIS	33	29
DUODENAL ULCER	10	10
GASTRIC ULCER	2	1
TOTAL	109	90

82.6% of the cases (n=109) were positive by Rapid urease test.

Detection of *H.pylori* by Culture (n-109)



23.9% (n=109) of the cases were positive by culture

DISCUSSION

H.pylori is responsible for one of the world's most common bacterial infections. The significant role of *H.pylori* in the etiology of gastric disease is now undisputed. Many factors like low socioeconomic conditions such as overcrowding, poor sanitation, close contact with infected persons, food habits, environmental factors, smoking and alcohol consumption appear to be associated with colonization and infection of H. pylori in humans.

The present cross sectional study was conducted to know the prevalence of H. pylori in Alcoholic and non-alcoholic gastritis patients in our population of SBMCH, Chennai.

This study is based on using conventional methods for detecting H. pylori infection. Biopsy based tests namely rapid urease test, histopathalogical examination, bacterial culture and sensitivity were used. The conclusion from the study gives correlation of the conventional methods with endoscopic diagnosis and the association of risk factors with H. pylori positivity.

This study included a total of 109 patients (presenting abdominal pain as the predominant symptom) in alcoholic and non-alcoholic gastritis patients.

All the 109 samples were subjected to RUT, Gram staining, Giemsa staining, Histopathological study, Culture and Sensitivity.

Out of 109 samples studied by RUT, 90 (82.6%) were positive

Culture positivity in the present study was 23.9% (Figure 5) Arora et al89 reported cultural positivity of 28%. The low isolation rate may be due to the distribution of H. pylori in gastric mucosa, its fastidious nature, administration of antibiotics (for other infection) and PPI(39). Anti-microbial susceptibility was performed by disc diffusion method and the isolates were susceptible to all the antimicrobials tested (amoxicillin, clarithromycin, metronidazole, ciprofloxacin, erythromycin and tetracycline) in the present study.

RUT showed about 79% sensitivity and 88% specificity.

Culture for H. pylori was positive only in 23.9% of the cases

CONCLUSION

The simple and inexpensive Rapid Urease Test and Giemsa stain detected the maximum number of positive cases.

Culture for H. pylori was low, revealing that isolation of H. pylori by culture is possible in reference laboratories.

In general, *H. pylori* have become the commonest infection associated with gastrointestinal diseases. This may be due to the advanced, accurate and more rapid diagnostic facilities available now. The most simple, rapid and inexpensive tests like RUT and Giemsa staining can be considered along with the endoscopic clinical findings to diagnose H. pylori infection and H. pylori eradication therapy can be started and followed up to prevent further complications and development of gastric carcinoma.

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