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Original Article

Isolation of *Helicobacter Pylori* from Gastric Biopsy Specimens and Evaluation of Common Contaminants Associated with *H. Pylori* Cultures

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ABSTRACT

Introduction: *Helicobacter pylori* infection is regarded as the causal factor of most of the diseases of the gastrointestinal tract in adults and children. Isolation of *H. pylori* by culture is highly specific and is considered as the gold standard technique. But often *H. pylori* are difficult to isolate in proper culture media.

Aim: The present study aims to isolate *H. pylori* in a non selective culture medium and review the common contaminants associated with it.

Methods and Materials: A cross-sectional hospital-based study was undertaken between 2014 and 2015 where 100 gastric biopsy specimens were collected via upper gastro-intestinal tract endoscopy from suspected gastritis, gastric ulcer disease and gastric carcinoma patients and cultured on a non-selective medium supplemented with 7% sheep blood. The common culture contaminants found on the media plates were reviewed. Rapid urease test of the specimens were also performed.

Results: *H. pylori* were isolated by culture from nine specimens out of 100. Rapid urease tested positive for 75 specimens. The culture contaminants were identified as *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter species, Escherichia coli, Bacillus* species and *Pseudomonas aeruginosa.* The *H. pylori* isolates were preserved in 20% glycerol at *-80ºC* for further analysis.

Discussion and Conclusion: Contamination of the culture media rendered low rate of isolation of *H. pylori* as compared to detection by rapid urease test, leading to false negative results and affecting the sensitivity and specificity of the culture techniques.

KEYWORDS: Culture, culture contaminants, *Helicobacter pylori*, Endoscopy, Rapid urease test.

INTRODUCTION

Helicobacter pylori is regarded as an important causative agent of majority of the diseases of the upper gastrointestinal tract such as peptic ulcer, duodenal ulcer and gastric cancer in adults as well as in paediatric patients. *H. pylori* infection can be diagnosed by both endoscopic methods such as rapid urease test, culture, histopathology etc., as well as non- endoscopic methods such as urea breath test, stool antigen test etc[1]. Isolation of *H. pylori* from gastric biopsy specimens by culture is highly specific, provides the greatest yield of *H. pylori* and is regarded as a prerequisite for antibiotic susceptibility testing as well as detailed characterisation of isolates.[2,3]

Isolation of *H. pylori* by culture is often difficult to perform mainly due to its micro-aerophilic requirements, fragile nature of the organism, unavailability of adequate transport media and presence of contaminants in the culture medium[4]. In 2012, Yuan Hu et al. demonstrated the high prevalence of non *H. pylori* bacterial flora in gastric biopsies concurrent with *H. pylori* infection[5]. The common culture contaminants may be *Staphylococcus* spp., *Bacillus* spp, *Candida* spp, *Klebsiella pneumoniae* and *Pseudomonas aeroginosa* and other environmental bacteria, oropharyngeal flora of the stomach and intestinal bacteria[6,7].

Although accurate statistics regarding the rate of contamination of biopsy specimens are unavailable, several studies have shown the influence of transport conditions and media on isolation of *Helicobacter pylori*[8]. In 1996, Hulst et al. reported effect of specimen

collection techniques, transport media, and incubation of cultures on the detection rate of *H. pylori*[9].

The present study was conducted to isolate *H. pylori* in a non-selective culture media contaminated with bacteria other than *H. pylori* and review the common contaminants (bacteria other than *H. pylori*) found on *H. pylori* culture plates.

MATERIALS AND METHODS

Patients of all age groups attending our hospital and undergoing upper gastrointestinal endoscopy for suspected gastritis, gastric ulcer disease and gastric carcinoma were included in the study as cases. All cases were included after obtaining written consent from themselves or their guardians (in case of minors).

Study Design

A cross sectional hospital based study was undertaken between 2014 and 2015 where a total of 100 gastric biopsy specimens from patients of all age groups with gastritis, gastric and duodenal ulcers and gastric carcinoma were collected via routine upper gastro-intestinal tract endoscopy. Two antral biopsy specimens were taken from each patient, placed in phosphate buffered saline and transported to the laboratory immediately. All cases were included after obtaining written consent from themselves or their guardians (in case of minors). Present study was approved by the Institutional Ethics Committee (Human) of Assam Medical College, Dibrugarh prior to initiation of the work and was carried out according to the guidelines of the Committee.

Isolation and identification of *H. pylori* and other bacteria

Out of the two biopsies, one was transferred to the rapid urease test kit (CLO Test) for determining the presence of urea. The other biopsy was crushed manually and a loopful of tissue homogenate was cultured on a non-selective medium such as Tryptic Soy Agar (TSA) (BD-Difco) supplemented with 7% sheep blood. The plates were incubated in a micro-aerophilic environment (Anoxomat MART Microbiology) containing 5-7% O_2, 10-12% CO_2, 6-7% H_2 and 75-80% N_2. The plates were observed from day 3 upto day 8. Suspected *H. pylori* colonies and other bacteria of different colony morphology observed on the culture plates were subcultured using the same media and culture conditions. Gram staining and biochemical tests of all bacteria including suspected *H. pylori* colonies for the production of enzymes urease, oxidase and catalase were performed. Biochemical tests specific for other bacteria present on the plates were also performed on the basis of observation of their Gram staining morphology.

RESULTS

In the present study, out of the 100 specimens cultured, 75 tested positive for *H. pylori* in rapid urease test. However *H. pylori* could only be isolated by culture from 9 specimens due to potential contamination of the culture plates with bacteria other than *H. pylori*. (Table 1) Colonies of *H. pylori* appeared small, round and translucent on TSA. These nine culture isolates were confirmed by observing their Gram negative spiral morphology and positive urease, oxidase and catalase test. These were then preserved in 20% glycerol at -80ºC for future analysis.

Seventy five percent of the specimens cultured on the agar plate revealed a mixed bacterial population consisting of three or more different types of colonies. From this population, individual colonies of different colony morphology were picked up and subcultured on TSA. The colonies were Gram stained and their colony morphology was observed along with biochemical tests specific for that bacteria. (Table 2) Out of the 100 specimens, the different types of organisms (contaminants) found on the culture plates are summarised in Table 2 and Figure 1.

More than one type of organism was observed on each culture plate. The plates that showed the presence of *Staphylococcus aureus*, also revealed *Klebsiella pneumoniae* or *E. coli*. The plates that showed the presence of *Pseudomonas aeroginosa* also revealed *Bacillus* spp or *Acinetobacter* spp and so on.

Table 1: Comparison of Rapid Urease Test and Culture method in Isolation of *H. Pylori*

Rapid Urease Test Positive		Rapid Urease Test Negative	Total Biopsies collected
Culture Positive	Culture Negative		
9	66	25	100
75			

Table 2: Gram staining of the bacteria other than *H. pylori* and contaminants of the culture medium.

Contaminant	*Staphylococcus aureus*	*Klebsiella pneumonia*	*Pseudomonas aeroginosa*	*E. coli*	*Acinetobacter spp*	*Bacillus spp*
Gram stain	Gram Positive cocci	Gram negative bacilli	Gram negative bacilli	Gram negative bacilli	Gram negative coccobacilli	Gram Positive bacilli
% of contamination	40	40	35	30	25	20

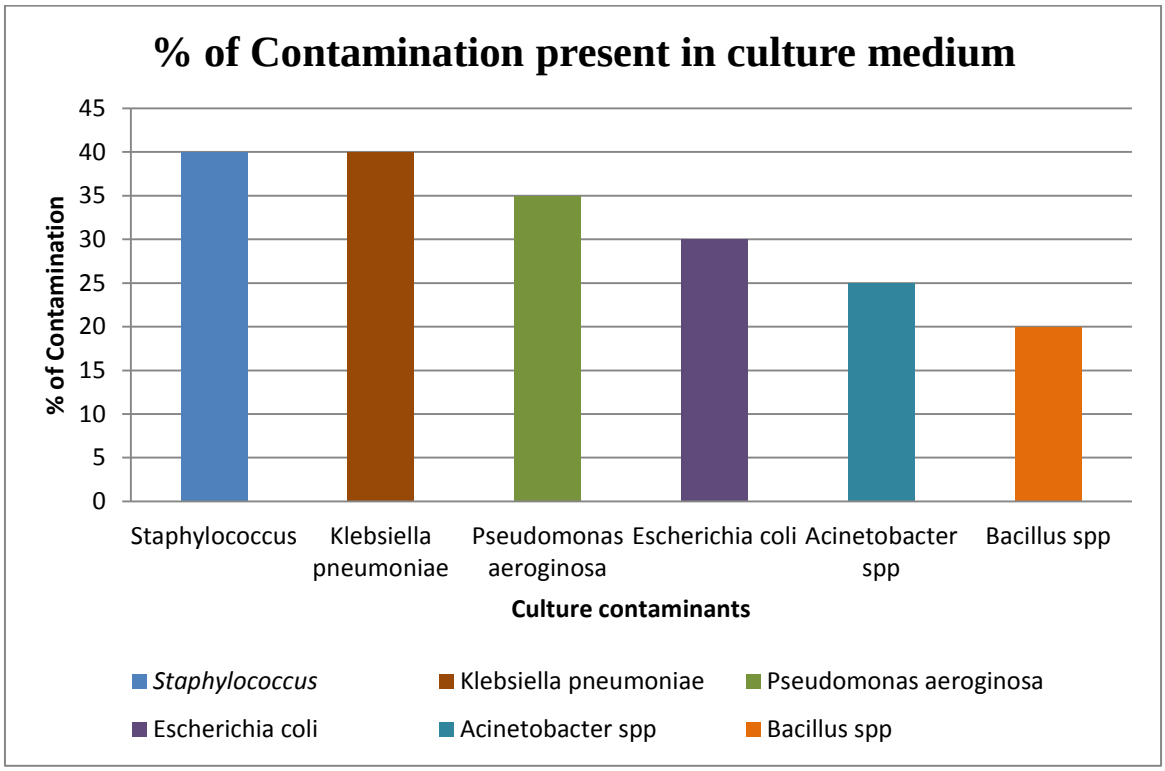

Figure 1: Chart showing % of contamination present in the culture medium.

DISCUSSION AND CONCLUSIONS

Helicobacter pylori is a major human pathogen which inhabits the mucous layer overlying the gastric epithelial cells in humans[10]. It is recognized by the World Health Organization to be the most important causal factor of gastric carcinoma as well as chronic gastritis and gastro duodenal ulcer disease.[10,11] Diagnosis of *H. pylori* infection by culture and histopathology are considered highly specific. We attempted to isolate this organism by culturing 100 biopsy specimens on blood based non selective media where 75 out of these 100 samples tested positive for *H. pylori* in Rapid Urease Test. Besides determining the presence of urea the rapid urease test was also done to determine the actual number of *H. pylori* positive specimens which could not be isolated by culture. But the sensitivity and specificity of the culture techniques were greatly affected by the presence of bacteria other than *H. pylori* on the culture media. A single culture plate revealed a mixed bacterial population consisting of two, three or more different type of organisms. For this reason, suspected *H. pylori* colonies were masked by the growth of these bacteria. The rate of isolation of *H. pylori* by culture was relatively low as compared to the rapid urease test which detects the presence or absence of the organism by detecting the production of the enzyme urease indicated by colour change from yellow to pink[10,12]. For this reason we were able to isolate only 9 positive *H. pylori* culture isolates among the contaminants. We have not yet used selective media such as Skirrow's supplement in the culture media since it was important for us to evaluate the extent of contamination of the plates and the different types of contaminants on it.

Contamination of the culture medium may occur due to;

a) Improper handling and transport of the specimens from the endoscopic suite to the laboratory[13]

b) Presence of a contaminant tissue fragment in the biopsy specimen which may appear during tissue processing[14]

c) Improper cleaning and disinfection of endoscopes[7,15]

d) Prolonged storage of the transported biopsy sample at ambient temperature[16]

e) Delay in processing, transport and culture of the biopsy sample[17]

The gastric mucosa of the human stomach harbours a wide range of normal microbial flora which may become opportunistic pathogens if the host defense mechanism fails[18]. This mostly comprises of organisms such as *Streptococci*, *Micrococci*, Enterobacteriaceae, yeasts and anaerobic Gram-positive cocci and rods. In 1998, Osato et al. demonstrated various microflora of gastric biopsies such as *Streptococci*, *Micrococci*, *Staphylococci*, Enterobacteriaceae and yeasts from patients with duodenal ulcer and gastric cancer[19]. According to their study, at least one type of organism with different colony morphology was present on the culture plate. These findings may be compared to the present study in which the plates consisted of two or more different type of organisms. In 2001, Song et al. developed a novel approach of reproducing better yields of *H. pylori* through culture from contaminated specimens[20]. This study suggested that exposing and pre-treating the patients' gastric biopsy as well as saliva specimens with hydrochloric acid (HCl) and urea facilitated the growth and isolation of *H. pylori*.

Although the above mentioned studies correlate with the present study and also recommends ways of improving *H. pylori* isolation, such kind of instances suggest that contamination of samples particularly associated with culture of fastidious bacteria such as *H. pylori* may pose as a growing concern for the microbiologists since such contamination would render false negative results and may lead to misdiagnosis[21].

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