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EVALUATION OF DIFFERENT TRANSPORT AND ENRICHMENT MEDIA FOR THE ISOLATION OF HELICOBACTER PYLORI

Farhat Rizvi and Abdul Hannan*

Department of Pathology Ayub Medical College, Abbottabad and * AFIP, Rawalpindi

Background: Helicobacter pylori are traditionally difficult to grow on culture media. The present study aims to determine the optimal transport and culture media for growing the microorganisms. ***Methods:*** One hundred antral biopsies were obtained in duplicate from patients complaining of upper GIT symptoms. Growth was obtained from 44% biopsies transported in Semi Solid Motility Medium (SSMM) and 42% from thioglycollate broth. This difference was not statistically significant (p=0.1). Brain Heart Infusion Agar (BHI) plus 7% Horse Red Blood Cells (HRBC) plus SR69 (antibiotic supplement) was found to be the best of three culture media for isolation of H. pylori, with a growth rate of 45% (p =<0.001). ***Results:*** This study shows that H. pylori is a common cause of gastro-duodenal disease in Pakistan. Proper transport and selection of culture media is required for optimal isolation from gastric biopsies.

INTRODUCTION

Helicobacter pylori is now established as one of the causative factors in gastritis and peptic ulceration [1-4]. The microbiological aspects of this microorganism have acquired great significance to further our knowledge about optimal methods of detection, identification and eradication.

In 1980 Warren noted that the majority of endoscopic biopsies from patients with gastritis and peptic ulceration were colonized with curved Campylobacter like organisms. Marshall and colleagues set up a prospective study and attempts were made to culture the microorganism. No growth was obtained until the 35th biopsy, which was left in the incubator due to Easter holidays, and examined after five days. A heavy growth of Campylobacter like microorganisms was found on the non-selective medium [5, 6].

These Campylobacter like organisms (first isolated and identified by Marshall and Warren in 1984) [5, 6] were first called Campylobacter pyloridis [7] and later renamed Helicobacter pylori [8]. Due to specific growth requirements, i.e., presence of horse blood or sheep blood and slow growth (3 – 5 days), the growth of this microorganism remained difficult. Isolation rate varied according to transport media and culture media used [9 - 13].

Keeping in view the suspected high prevalence of H. pylori infection and related gastritis in developing countries, including Pakistan, this study was designed to compare two transport media [a- Thioglycollate broth b- Semi-solid Motility Medium (SSMM)] and three culture media [a- Brain Heart Infusion Agar (BHI) plus 7% HRBC plus SR69 (Oxoid) b- Chocolate Agar c- Columbia Agar plus 7% HRBC plus SR69 plus SR84 (Oxoid)], for the growth and isolation of Helicobacter pylori.

Objectives of the Study:

1. To determine the prevalence of H. pylori infection in gastric biopsy specimens.
2. To determine the more suitable transport medium from the two available ones.
3. To determine the more suitable culture medium from the three available ones.

MATERIALS AND METHODS

Gastric antral biopsies from 100 patients complaining of upper GIT symptoms were obtained in duplicate.

Transport:

One of the biopsies was put in Thioglycollate broth and the other in SSMM. The specimens were processed within 4 hours of collection.

Culture:

All the 100 specimens were cultured on 3 culture media. The biopsy specimen was crushed in a sterile tissue grinder (Wheaton Scientific) and inoculated on 3 culture media:

1. Brain Heart Infusion Agar plus 7% HRBC plus SR69(Oxoid). SR69 (antibiotic supplement) contains:
    a. Vancomycin 5 mg
    b. Trimethoprim lactate 25 mg
    c. Polymyxin B 1250 I.U.
2. Chocolate Agar
3. Columbia Agar plus 7% HRBC plus SR69 plus SR84 (Oxoid). SR84 – growth supplement contains:
    a. Sodium pyruvate 0.125 g
    b. Sodium metabisulphite 0.125 g
    c. Ferrous sulphate 0.125 g

The plates were incubated under microaerophilic conditions at 37^0C, using an anaerobic jar and anaerobic GasPak kit without catalyst to generate a microaerophilic environment. Plates were inspected first on day 3 and then after one-day interval for a total of ten days. GasPak was changed whenever the jar was opened.

H. pylori was identified by the following criteria:

1. Colonial morphology, Motility test, Catalse oxidase and Urease Tests.
2. Sensitivity to Nalidixic acid and Cephalothin.
3. Growth on BHI and HRBC plus 40 mg/L triphenyl tetrazolium chloride [14].

Results were analysed by Chi-Square (χ^2) test analysis.

Table 1: Criteria for Identification of Helicobacter pylori

Gram Stain	Gram Negative Ox Bow, U Shaped Spiral Rods
Motility	Sluggishly Motile
Catalase	Positive
Oxidase	Positive
Urease	Very Strongly Positive Within 1-5 Minutes
Nalidixic Acid Sensitivity	Resistant
Cephalothin	Sensitive
Growth On BHI + HRBC + 40mg/L Triphenyl Tetrazolium Chloride	Golden Colonies

RESULTS

Comparison Between Transport Media:

Growth of H. pylori was obtained from specimens transported in SSMM in 44 cases out of 100, i.e., 44%. Growth occurred in specimens transported in Thioglycollate broth in 42 out of 100 cases , i.e., 42%. This difference was not statistically significant (p = 0.1, Table – 2).

Table-2: Comparison between transport media

Transport Media	Total	Growth Of H. Pylori
Thioglycollate Broth	100	42 (42%)
Semisolid Motility Medium	100	44 (44%)

Comparison Between Culture Media:

In our study, BHI plus 7% HRBC and SR69 proved to be the best medium; growth occurred in 45 cases out of 100, i.e., 45%. The second medium was Chocolate Agar; growth occurred in 18 out of 100 cases, i.e., 18% of cases. The third medium was Columbia Agar plus 7% HRBC plus SR69 plus SR84; growth occurred in only 10 out of 100 cases, i.e., 10%. The difference between the culture media was found to be statistically significant (p = <0.001).

Table 3: Comparison Between Culture Media

Culture Media	Total	Positive
BRAIN HEART INFUSION AGAR + 7% HRBC + SR69 (OXOID)	100	45 (45%)
CHOCOLATE AGAR	100	18 (18%)
COLUMBIA AGAR + 7% HRBC + SR69 + SR84 (OXOID)	100	10 (10%)

SR69: (Antibiotic Supplement)
SR84: (Growth Supplement)

DISCUSSION

Although presence of microorganisms in the gastric mucosa has been observed since the last century (cited by Rathbone et al) [6, 15], success in culture and isolation was only obtained in 1984, due to the fastidious and slow growing nature of this microorganism.

Great care is needed in the collection, transport and culture of this microorganism. Collection procedure and transport of the biopsy specimens has been found to be a very important factor in the successful growth of this microorganism.

In this study, the rate of isolation from the two transport media was nearly the same. This is in contrast to higher recovery rates of Campylobacter spp. Isolated from SSMM in a previous study [16]. This may be due to the fact that the above study was not carried out specifically for H. pylori, which has been found to be different from other Campylobacter spp [8].

Sterility of forceps and time taken between collection of the biopsy and inoculation of the specimen on different media was found to be more important in the isolation rate of H. pylori. The specimens were transported within 4 hours of collection; if delay of more than 4 hours occurred, the biopsy was placed at 4^0C for a few more hours. When delay occurred, the specimens were contaminated with Pseudomonas spp. It is therefore obvious that collection procedures and transport times are more important factors than type of transport medium used. Even sterile saline has been used to transport the biopsy successfully [3]. However some sort of transport has to be used to prevent drying of the biopsy and also because H. pylori is microaerophilic and may die due to exposure to air.

After reaching the laboratory the biopsy was crushed in a tissue grinder; this method was found to be superior to rubbing the biopsy on culture plates. Gram stain of crushed tissue was tried but recognition of the microorganism was

difficult due to much background material. Rinse imprint method and Gram stain has been found to be a very sensitive method of detection of H. pylori by previous workers [17].

In this study, BHI plus 7% HRBC plus SR69 was the most effective culture medium with a positivity rate of 45%. This was because of the rich medium and antibiotic supplement used to suppress contaminants. A higher rate as reported previously [10 - 12] was not obtained most probably due to patient selection. Patients with upper GIT symptoms were included, the age varying from 22 – 65 years. Also for maximum detection the patients must be off treatment when biopsy is taken [18]. In our study the patients' biopsies were taken without any prior instructions to stop medication.

Columbia Agar plus 7% HRBC plus SR69 plus SR84 contained the same antibiotic supplement, but growth supplement SR84 was also added; growth was poor and only 10% isolation rate was obtained. This was most probably due to the inhibitory effect of sodium metabisulphite present in the growth supplement SR84 as reported by Goodwin et al [11]. It was primarily used because it is a good growth supplement for other Campylobacter spp.

Chocolate Agar has been used successfully in many studies [3, 6]; when used with antibiotic supplement it is called Campy Chocolate Plate and has been found to be an effective medium [9]. The disadvantage of the Chocolate Agar used in the present study was lack of antibiotics supplements, so that overgrowth of contaminants was found to be a problem in many cases. Horse or Sheep blood in the culture medium is an essential growth requirement. Human blood should not be used, as it may be inhibitory due to the presence of antibodies and other nonspecific factors, complement, antibiotics, etc. [19].

The problem we faced with our culture media was fungal contamination due to prolonged incubation. Therefore use of an antibiotic supplement with antifungal agents could be more effective. An antibiotic supplement SR147 (Oxoid) may be used, which contains Vancomycin, Trimethoprim, Cefsuldin and Amphotericin B. Columbia Agar base and laked horse blood (SR48 Oxoid) has been recommended with this supplement [18]. This study thus shows that Helicobacter pylori are indeed present in the gastric mucosa in patients with gastroduodenal disease in Pakistan. Culture is the most specific test, as it is a direct evidence of the presence of the micoorganism. However due to difficulty in culture, other tests may be used to ascertain true prevalence in Pakistan. Culture is important to study the characteristics and antibiotic sensitivity pattern for treatment. For optimal isolation of H. pylori, patient selection, collection procedure, transport time and use of suitable enrichment media with antibiotic supplement and microaerophilic environment are the most important factors to obtain growth of this microorganism.

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