COMPARISON OF MEDIA AND ANTIBIOTIC SUPPLEMENTS FOR ISOLATION OF *HELICOBACTER PYLORI* FROM GASTRIC BIOPSIES

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Abstract. Our objective was to improve the media and the antibiotic supplements in order to increase the detection rate of *Helicobacter pylori* from gastric biopsy specimens. For the primary isolation of *H. pylori* taken from gastric biopsies, we compared the efficacy of two media: Columbia blood agar (CBA, Difco); brain heart infusion agar (BHIA, Difco); and two antibiotic supplement sets – a commercial antibiotic supplement (SR147, Oxoid) and an in-house antibiotic supplement (IHS). Gastric biopsies obtained from 210 patients were diagnosed by culture, rapid urease test (RUT) and histology. The true positive criteria were defined as a culture or both urease and histology tests being positive. The *H. pylori* infection rate was 44.3% (93/210). To compare the two media, a total of 106 gastric biopsies were plated on CBA or BHIA with 7% human blood, containing the antibiotic supplement SR147 and incubated under microaerophilic conditions. Of the 106 samples, 48 (45.3%) case of *H. pylori* infection, compared to the true positive criteria. The isolation rate using a combination of the two media was 83% (40/48). Of the 40 samples, 36 (90%) and 35 (87.5%) were positive on CBA and BHIA, respectively. To compare the two antibiotic supplement sets, a total of 104 gastric biopsies were plated on CBA, containing the commercial antibiotic supplement SR147 (5 mg/l trimethoprim, 10 mg/l vancomycin, 5 mg/l amphotericin B and 5 mg/l cefsulodin) or containing IHS (5 mg/l trimethoprim, 10 mg/l vancomycin, 2 mg/l amphotericin B and 2,500 U/l polymyxin B). Of the 104 samples, 45 (43.2%) case of *H. pylori* infection were found compared to the true positive criteria. The isolation rate using a combination of the two selective supplement sets was 82% (37/45). Of the 37 samples, 35 (95%) and 34 (92%) were positive with SR147 and IHS, respectively. Our study indicates that the combination of the two media and two antibiotic supplements is useful for maximum recovery of *H. pylori* isolated from gastric biopsies. CBA, and the commercial antibiotic supplement SR147 provided higher detection rates for *H. pylori* than BHIA, and IHS but the differences were not statistically significant.

INTRODUCTION

Helicobacter pylori is a gram-negative, spiral shaped, microaerophilic bacteria that causes gastritis, peptic ulcers and gastric cancer (Heatley, 1995; Dunn *et al*, 1997). These observations explain the interest of investigators in developing accurate diagnostic methods. *H. pylori* diagnosis consists of invasive and non-invasive methods (Dunn *et al*, 1997; Logan and Walker, 2001). Culture, an invasive method, is not usually recommended for routine diagnosis of *H. pylori* infection because it is a tedious, time-consuming and an expensive procedure, requiring skilled personnel.

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However, it is accepted as the gold standard for bacteriologists and is a prerequisite for further studies of the organism, such as antibiotic resistance monitoring, strain classification, typing, and other comparative research (Hazell, 1993; Heatley, 1995; Dunn *et al*, 1997; Megraud, 1997; van Doorn *et al*, 2001).

Culturing on solid media is the standard technique used in most laboratories for the isolation of *H. pylori* from gastric biopsy specimens (Hazell, 1993; Piccolomini *et al*, 1997; Stevenson *et al*, 2000). A large number of different agar media for recovering *H. pylori* have been described (Piccolomini *et al*, 1997; Stevenson *et al*, 2000; Ding *et al*, 2001). There are two main types of media : 1) nonselective media based on nutrient agar, such as brain heart infusion agar, blood agar, or brucella agar supplemented with 5% to 10% sheep or horse blood, or soluble starch, and 2) selective media, based on supplemented nutrient agar containing antibiotics (Buck and Smith, 1987; Dent and McNulty, 1988; Goodwin *et al*, 1985; Krajden *et al*, 1987).

Many laboratories, however, have found the primary isolation of *H. pylori* from gastric biopsies is still problematic (Tee *et al*, 1991; Hachem *et al*, 1995; Heatley, 1995; Lerang *et al*, 1998). Although, success in *H. pylori* isolation and growth depends on many factors, such as the method of specimen collection, time, procedure for tissue processing, composition of culture media, including growth supplements and antibiotic supplements, and environment (CO_2 and humidity) (Tee *et al*, 1991; Hazell, 1993), the media and antibiotic supplements are the main requirement for successful *H. pylori* isolation (Dent and McNulty, 1988). Some selective media are expensive and are not used in routine laboratories in Thailand, therefore, the selection of appropriate media used for *H. pylori* primary isolation should be established.

Owing to the high rate of contaminants on nonselective media, media with antibiotics were thought to be mandatory for the detection of *H. pylori* (Goodwin *et al*, 1985). A popular antibiotic supplement commercially available is SR 147 (Hazell, 1993). However, some studies have found the commercial supplement gives variable numbers of *H. pylori* isolation (Tee *et al*, 1991). Therefore, an in-house supplement (IHS) with a different antibiotic composition was evaluated.

One objective of this study was to compare two media popularly used in laboratories: Columbia blood agar (CBA) and a brain heart infusion agar (BHIA). The second objective was to compare two antibiotic supplement sets: SR147 (Oxoid) and IHS, for the primary isolation of *H. pylori* from gastric biopsies.

MATERIALS AND METHODS

Patients and endoscopy

Two hundred ten consecutive dyspeptic patients who underwent upper gastrointestinal endoscopy were recruited from the Endoscopy Unit of Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Thailand. Informed consent was obtained from each patient before being included in this study. Three gastric mucosal biopsy specimens from the antrum and the corpus were obtained from each patient and divided into three parts. Both antral and corpus specimens were used for culture, the rapid urease test (RUT) and histological examination.

Culture

The culture was performed according to Hazell *et al* (1989) with modification. Briefly, each antral and corpus specimen was immediately placed into transport media and brought to the laboratory within 2 hours, and stored under cold conditions. Two biopsy specimens were homogenized in 200 µl of sterile normal saline.

For a comparison of the two media, a total of 106 gastric biopsies were plated on Columbia blood agar (CBA, Difco, Detroit, Michi-

gan, USA) with 7% human blood and on brain heart infusion agar (BHIA, Difco) with 7% human blood. Both media contained the antibiotic supplement SR147 (5 mg/l trimethoprim, 10 mg/l vancomycin, 5 mg/l amphotericin B, 5 mg/l cefsulodin; Oxoid, Unipath Ltd, Basingstroke, Hamshire, England).

For a comparison of the two antibiotic supplements, a total of 104 gastric biopsies were plated on CBA, containing the supplements SR147 or IHS (10 mg/l vancomycin, 5 mg/l trimethoprim, 2,500 U/l polymyxinB, 2 mg/l amphotericinB) and incubated at 37ºC under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2) and examined at 4 and 7 days of incubation. Characteristic colonies of *H. pylori* were confirmed by Gram staining, oxidase, catalase and urease tests.

Commercial rapid urease test (RUT, Pronto Dry test)

The RUT was performed according to the manufacturer's instructions (Medical Instruments Corporation, Solothurn, Switzerland). Briefly, one antral and one corpus specimen were directly inoculated onto the commercial RUT agar gel. The results were observed and recorded at 24 hours. A positive was indicated when the color changed from yellow to pink.

Histological examination

One antral and one corpus biopsy were each fixed in 10% buffered formalin, processed, then embedded in paraffin. Four sections 3-4 microns thick were stained with modified Warthin-Starry stain for identification of *H. pylori* (Cohen and Laine, 1997; Li *et al*, 2004). The presence of spiral organisms on any of the slides was considered positive for *H. pylori.*

True positive criteria

The criteria for a true positive for *H. pylori* was defined as either 1) a positive culture or 2) a negative culture but a positive result on both the RUT and histological examination (Pajares-Garcia, 1998; Liao *et al*, 2003).

RESULTS

Gastric biopsies, obtained from 210 patients, were diagnosed by culture, urease test and histology. Comparison of CBA and BHIA indicated the prevalence of *H. pylori* infection was 45.3% (48/106) according to the positive criteria. The isolation rate using a combination of the two media was 83.3% (40/48) whereas it was 91.6% (44/48) by RUT and 97.9% (47/48) by histological examination (Table 1). Of the 40 samples with growth on the two media, 36 (90%) and 35 (87.5%) were positive on the CBA and BHIA, respectively. Five (12.5 %) and 4 (10%) of 40 samples were positive on the CBA or BHIA alone, respectively (Table 2).

For a comparison of the two antibiotic supplement sets, *H. pylori* infection was found in 43.3% (45/104) compared to the positive criteria. The isolation rate using a combination of the two antibiotic supplement sets was 82% (37/45). Of the 37 samples, 35 (95%) and 34 (92%) were positive with the SR147 and IHS, respectively. Of the 35 samples positive with SR147, 3 (8.1%) were found on SR147 alone, and of the 34 samples positive with IHS, 2 (5.4%) were found on IHS alone (Table 3).

DISCUSSION

Helicobacter pylori is both microaerophilic and a fastidious microorganism, because it requires a nutrient rich medium and an atmosphere enriched in CO_2 (Hazell, 1993). Primary isolation of *H. pylori* from gastric biopsy specimens is a difficult process for routine laboratories. The sensitivity of isolation in specialized laboratories varies widely, ranging from 75 to 90% (Leung and Sung, 1996; Logan and Walker, 2001; Kisa *et al*, 2002). This may be due to the fastidious nature of *H. pylori* and a number of factors that are hard to control, such as patchy distribution of the organism on the gastric mucosa, contamination of biopsy forceps and/or the loss of viability of the

Table 1
Comparison of two culture media, urease test and histology of gastric biopsies obtained from 106 patients.

Culture medium		Rapid urease test	Histology	Total No. (%)	Evaluation infection
CBA	BHIA				
+	+	+	+	29 (27.4)	TP*
+	-	+	+	4 (3.8)	TP*
-	+	+	+	2 (1.9)	TP*
+	+	-	+	2 (1.9)	TP*
-	-	+	+	8 (7.5)	TP*
-	+	-	+	1 (0.9)	TP*
-	+	+	-	1 (0.9)	TP*
+	-	-	+	1 (0.9)	TP*
-	-	+	-	2 (0.9)	FP
-	-	-	+	32 (30.2)	FP
-	-	-	-	24 (22.6)	TN
			Total	106 (100%)	48 (45.3)

TP = True positive, TN = True negative, FP = False positive
TP, culture positive or both urease and histological examination positive

Table 2
Comparison of two media, CBA and BHIA for isolation of *H. pylori.*

CBA	BHIA	Total No. (%)
+	+	31 (77.5)
+	-	5 (12.5)
-	+	4 (10)
Total		40/48 (83.3)

Table 3
Comparison of two antibiotic supplement sets, SR 147 (Oxoid) (5 mg/l trimethoprim, 10 mg/l vancomycin, 5 mg/l amphotericin B and 5 mg/l cefsulodin) and IHS (5 mg/l trimethoprim, 10 mg/l vancomycin, 2 mg/l amphotericin B and 2,500 U/l polymyxin B).

SR 147	IHS	Total No. (%)
+	+	32 (86.5)
+	-	3 (8.1)
-	+	2 (5.4)
Total		37/45 (82.2)

organisms during transportation, etc. Those reasons together resulted in a poor negative predictive value associated with culture of *H. pylori* (Hazell, 1993; Leung and Sung, 1996; Megraud, 1996). Although, culture has been considered the "gold standard" for the diagnosis of *H. pylori* infection by various investigators because of its 100% specificity (Dunn *et al*, 1997; Megraud, 1997; Logan and Walker, 2001), culture is now only usually used in the research setting. Thus, the need persists for a high *H. pylori* recovery rate from gastric biopsy specimens and a practical clinical treatment of *H. pylori* positive dyspeptic patients with gastritis and ulcers (Megraud, 1997).

Relatedly, resistance of *H. pylori* to metronidazole and macrolides has emerged worldwide, including in Thailand, and now constitutes a major problem in therapy (Wongkusoltham *et al*, 2001; Boyanova *et al*, 2002). These indicate the increasing use of culture in testing for *H. pylori* infection because it is the only diagnostic method that allows us

to assess the susceptibility of this microorganism to antimicrobial agents. In addition, the detection of low numbers of bacteria after antibiotic treatment may also require culture (Boyanova *et al*, 2002). We, therefore, anticipate that sensitivity testing will be required in cases of treatment failure or before initiating therapy in cases of patients harboring *H. pylori* infection (Megraud, 1997; Logan and Walker, 2001).

Comparison of CBA and BHIA in our study showed that CBA provided a higher detection rate than BHIA, but there was no significant difference. In addition, growth of *H. pylori* was easier on CBA than BHIA because *H. pylori* presented in greater numbers on CBA than BHIA (data not shown). Difference in the components of CBA and BHIA may be attributable to the growth of *H. pylori* (Hazell, 1993). Both media have rich basal medium. CBA is comprised of beef heart digest, pancreatic digest of casein, yeast extract, proteose peptone, corn starch, sodium chloride and agar, whereas BHIA is comprised of calf brain, beef heart, proteose peptone, dextrose, sodium chloride, disodium phosphate and agar (Zimbro and Power, 2003). However, no data has been reported how each of the components of each media promoted growth of *H. pylori* and these should be studied further. A previous report, however, showed that BHIA was the best medium, compared to trypticase soy agar (TSA), egg yolk agar (EYA) and Columbia blood agar-cyclodextrin (CBA-cd), (Hachem *et al*, 1995). By contrast, Chantrakooptunggool *et al* (1996) found that CBA was better than TSA.

In this study, we found that one medium used alone did not result in a maximum yield of *H. pylori*. A combination of two media is required for the maximum recovery of *H. pylori* from biopsy specimens. Some researchers have stated the use of a combination of more than two media should not be used because of the material and labor costs involved in processing these media. In addition, *H. pylori* is a fastidious microorganism and requires prompt processing of biopsy samples (Tee *et al*, 1991). The best medium for isolating *H. pylori* includes fresh blood and adequate humidity throughout incubation (Hazell, 1993). We used fresh human blood instead of horse or sheep blood because the use of human blood can reduce costs and human blood agar supports better growth than sheep blood agar (Chantrakooptungool and Kanjanahareutai, 1996).

As for antibiotic supplements, the composition of antibiotics which were different between SR147 and IHS were cefsulodin and polymyxin B. Cefsulodin is in SR147 and polymyxin B is in IHS. The difference in the antibiotic supplements in the media may influence the detection of *H. pylori* in biopsy specimens.

Dent and McNulty (1988) developed a modified Skirrow's medium where cefsulodin (5 mg/l) was substituted for polymyxin B, and amphotericin B (5 mg/l) was added to inhibit *Candida* spp, a common contaminant of stomach biopsy specimens. This antibiotic supplement is now commercially available as SR147. It was claimed that it can support the growth of all strains of *H. pylori* tested and greatly improved the isolation of *H. pylori* and could be used without a nonselective medium (Dent and McNulty, 1988; Hazell, 1993). Others have reported that the medium of Dent and McNulty is less efficient than Skirrow's original formation, which contained the polymyxin B (Tee *et al*, 1991). Our results agree with Dent and McNulty (1988) since cefsulodin yielded a higher detection of *H. pylori* isolation than polymyxin B, since we found SR147 had a higher detection rate than IHS, albeit without any statistically significant difference.

The difference in the kind of media and the components of the antibiotic supplements may influence the different strains of *H. pylori* growing in the media (Hartzen *et al*, 1997; Bertram-Drogatz *et al*, 1999; Scherer *et al*, 2003). Therefore, using the combination of two

media and two antibiotic supplement sets should increase the number of *H. pylori* strains from gastric biopsies.

We conclude that CBA, and the commercial antibiotic supplement SR147 provided higher detection rates for *H. pylori* than BHIA, and IHS, but the differences were not statistically significant. The use of a combination of the two media and two antibiotic supplement sets is useful for the maximum recovery of *H. pylori* by primary isolation and will ensure that a minimum numbers of isolates are missed.

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