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Evaluation of Selective and Nonselective Media for Isolation of *Helicobacter pylori* from Gastric Biopsy Specimens

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Abstract: The aim of the present study was to compare six media, three selective and three nonselective media, to determine the best combination of media for the primary isolation of *Helicobacter pylori*. Over a period of 8 months, mucosal antral biopsy specimens were obtained from 97 dyspeptic patients undergoing endoscopy. Biopsy samples were plated in parallel on all six media. Egg yolk emulsion agar (EYE), Skirrow's medium and modified Thayer-Martin medium were used as selective media; modified chocolate agar (MCHOC), Triptycase Soy Agar (TSA) and brain heart infusion agar were used as nonselective media. Overall, by using these six media, *H. pylori* were recovered from biopsy specimens from 48 of 97 patients, yielding an isolation rate of 49%. Comparison of all possible combinations of the six media showed that the highest rate of isolation of *H. pylori* was 100% (48 of 48) with EYE-MCHOC, followed by 97% (47 of 48) when EYE-SK was used. Conversely, it was found that none of the media used alone yielded a 100% rate of recovery (the maximum recovery rate was 92%, which was achieved with EYE). These results indicate that the association of EYE and MCHOC yielded the maximum recovery of *H. pylori* from gastric biopsy specimens. Therefore, the use of selective and nonselective media in parallel offers optimal recovery rates with only a slight increase in costs.

Key words: *Helicobacter pylori*, diagnosis, media, selective, nonselective

INTRODUCTION

Since the first description by Warren and Marshall (1983), *Helicobacter pylori* have been recognized as an important gastroduodenal pathogen. *H. pylori* infection is one of the most frequent bacterial infections in the world and this organism has been acknowledged to be a crucial factor in several different diseases ranging from gastritis to gastric malignancies (Valle and Gisbert, 2001; Chang *et al.*, 1999). *H. pylori* growths in spiral and coccoid forms in the human gut (Hua and Ho, 1996; Sato *et al.*, 2003). These observations explain the interest of investigators in developing accurate diagnostic methods. The isolation of *Helicobacter pylori* is useful for analyzing both genotype and antibiotic sensitivity (Rautelin *et al.*, 1997) and the culture of gastric mucosal tissue is the most specific method for diagnosing infection (Grove *et al.*, 1998). However, culture sensitivity is highly variable (60-90%) (Thijs *et al.*, 1996), attributable to such methodologic factors as biopsy site, transport medium, time from sampling to processing, culture medium and incubation conditions (Siu *et al.*, 1998). Over the years, there have been a number of reports on the media

that can be used for the successful culture of *H. pylori*. Originally, Marshall *et al.* (1984) used brain heart infusion chocolate agar supplemented with 7% horse blood. Subsequently, a variety of media, selective and nonselective, or a combination of both have been proposed for use in the primary isolation of *H. pylori* (Hachem *et al.*, 1995; Tee *et al.*, 1991), but the optimal method of recovery still remains to be established. Culturing on solid media is the standard technique used in most laboratories for the isolation of *H. pylori* from gastric biopsies. Selective media, such as Skirrow's medium, are required for the isolation of this organism from biopsies in which contaminating bacteria are present.

The objective of this study was therefore to compare and evaluate selective and nonselective media in order to determine the combination of media yielding the optimal recovery of *H. pylori* from antral mucosal biopsy specimens.

MATERIALS AND METHODS

Isolation and identification: The strains of *H. pylori* were isolated from patients attending the endoscopy suites,

who were referred for evaluation of upper gastrointestinal symptoms at December 2002 to May 2003. Protocols for collection, transport, culture and identification of *H. pylori* were followed at the Institute Pasteur of Iran. Three gastric mucosal antral biopsy specimens were collected from each of 97 dyspeptic patients (63 male and 34 female) undergoing endoscopy. Gastric biopsy specimens were placed in sterile tubes containing 1 mL of saline buffer and were stored at 4°C. The delay between the removal of the specimens and the inoculation onto culture media did not exceed 6 h. One biopsy specimen was used for rapid urease test. The other two-biopsy specimens for culture were thorough ground with a mortar and pestle in 1 mL of saline. To ensure equal distribution of tissue fragments, the homogenate was flushed up and down in a sterile, disposable plastic pipette and then was placed for isolation onto each of selective and nonselective media. The plates were incubated at 37°C in a microaerobic atmosphere (Campy Pak, Becton Dickinson) for up to 7 days. *H. pylori* was identified by Gram staining of suspect colonies and the presence of characteristic curved gram-negative bacilli and testing for the presence of urease, oxidase and catalase.

Media: Six different media were compared for primary isolation of *H. pylori*. Three selective media were used. EYE (Oxoid) contained Columbia agar, 10% egg yolk emulsion, 1% isovitalax and 40 mg of triphenyltetrazolium chloride (Sigma) per liter with an antibiotic supplement (5 mg of cefsulodin, 5 mg of trimethoprim, 6 mg of vancomycin and 6 mg of amphotericin B per liter). Modified Thayer-Martin medium (MTM) contained GC agar base, 10% soluble hemoglobin powder and GC supplement (10 g of yeast extract, 1.5 g of dextrose, 0.15 g of NaHCO₃, 3 mg of vancomycin, 7.5 mg of colistin methane-sulfonate, 12,500 IU of nystatin and 5 mg of trimethoprim per liter). Skirrow's medium (SK) (Oxoid) consisted of Columbia agar, 7% laked horse blood, Campylobacter growth supplement and Campylobacter selective supplement (10 mg of vancomycin, 2500 IU of polymixin B and 5 mg of trimethoprim per liter). Three nonselective media were used. Trypticase Soy Agar (TSA) consisted of Trypticase soy agar and 5% sheep blood. Brain Heart Infusion Agar (BHIA) contained brain heart infusion agar and 7% defibrinated horse blood. Modified chocolate agar (MCHOC) contained Columbia agar, 1% isovitalax and 5% sheep blood.

RESULTS

Over a study period of 8 months, 291 gastric mucosal biopsy specimens were obtained from 97 dyspeptic

Table 1: The isolation rate of *H. pylori* from gastric biopsy on selective and nonselective media

| Media | EYE | MCHOC | MTM | SK | TSA | BHIA |
|--------------------|-----|-------|-----|----|-----|------|
| Isolation rate (%) | 92 | 80 | 74 | 59 | 56 | 33 |

Table 2: The isolation rate of *H. pylori* from gastric biopsy on combinations of selective and nonselective media

| Combinations of media | Positive culture No. (%) |
|-----------------------|--------------------------|
| EYE+MCHOC | 48 (100) |
| EYE+MTM | 46 (95) |
| EYE+SK | 47 (97) |
| EYE+BHIA | 41 (85) |
| EYE+TSA | 46 (95) |
| MCHOC+SK | 42 (87) |
| MCHOC+MTM | 45 (94) |
| MCHOC+BHIA | 40 (83) |
| MCHOC+TSA | 40 (83) |
| MTM+SK | 39 (81) |
| MTM+BHIA | 35 (73) |
| MTM+TSA | 40 (83) |
| SK+BHIA | 30 (62) |
| SK+TSA | 39 (81) |
| TSA+BHIA | 33 (68) |

patients (63 males and 34 females) undergoing endoscopy. Overall, by using all six media, *H. pylori* was detected in 48 patients (29 males and 19 females), yielding an isolation rate of 49% (60.4% for males and 39.6% for females).

The growth rate of *H. pylori* on each of the selective and non selective media indicates in Table 1. None of the media by itself gave maximum recovery: EYE gave the highest isolation rate (92%), followed by MCHOC (80%), MTM (74%), SK (59%), TSA (56%), BHIA (33%). The recovery rate obtained with EYE was significantly higher ($p < 0.05$) compared with those obtained with MTM, SK, TSA and BHIA; the difference was not statistically significant for EYE compared with that for MCHOC. The recovery rates of *H. pylori* with all possible combinations of two media are presented in Table 2. The combination that appeared to be the most effective for the primary isolation of *H. pylori* was EYE (selective medium) plus MCHOC (nonselective medium), which yielded a maximum recovery rate of 100% (48 of 48) followed by EYE-SK (97%) and EYE+MTM (95%). Among the selective media, MTM gave the lowest contamination rate, a value significantly different from those obtained with SK, EYE, MCHOC, BHIA, TSA ($p < 0.05$). Among the nonselective media, BHIA gave the lowest rate, followed by MCHOC and TSA.

DISCUSSION

Primary isolation of *H. pylori* from a biopsy specimen is a difficult process: in specialized laboratories, isolation rates of 75 to 90% can be achieved (Goodwin *et al.*, 1985; Marshall *et al.*, 1985). This may be due to the fastidious

nature of *H. pylori* and to a number of factors that are hard to control (patchy distribution of the organism on the gastric mucosa, contamination of biopsy forceps, ingestion of anesthetic, presence of oropharyngeal flora, loss of viability of the organisms during transportation, etc.) and that are, altogether, responsible for a poor negative predictive value associated with culture of *H. pylori*. For these reasons, although culture has been considered the gold standard for the diagnosis of *H. pylori* infection by various investigators (Jones *et al.*, 1984; Malfertheiner and Pieramico, 1992; Taylor *et al.*, 1987), culture is now usually used only in the research setting. However, the need for a high *H. pylori* recovery rate from gastric biopsy specimens is increasing among practicing clinicians.

The results of the present study indicate that, among the six media tested, EYE is the most sensitive for the primary isolation of *H. pylori*, yielding a recovery rate of 92%. Present results conflict with those of Hachem *et al.* (1995). Furthermore, the red color induced by triphenyltetrazolium chloride included in EYE made the *H. pylori* colonies easier to spot on EYE than on the other media. The isolation rate of 59% yielded by SK is the same with another report (Piccolomini *et al.*, 1997) but not comparable to those reported in previous studies (Dent and McNulty, 1988; Krajden *et al.*, 1987; Tee *et al.*, 1991), which reported values ranging from 94 to 97%. It is possible that these differences could be a result of methodological variability, slight differences in medium components and the freshness of the media; differences between isolates from different geographical areas also possibly exist. Among selective media, MTM was the most selective, confirming that the antimicrobial supplement present in this medium (vancomycin, colistin, trimethoprim and nystatin) limits the overgrowth by flora of the upper respiratory tract (Parsonnet *et al.*, 1988).

Present modified chocolate agar yielded a good rate of 83%, comparable to those obtained with classic chocolate agar in other studies (Dent and McNulty, 1988; Glupczynski *et al.*, 1989; Tee *et al.*, 1991). In the primary isolation of *H. pylori*, it is very important to consider the qualitative growth of contaminants isolated on each medium. In fact, when the growth of contaminants was scanty, it did not interfere with *H. pylori* isolation. Conversely, growth of *H. pylori* could not be detected on the plate in the presence of a high number of contaminants. BHIA gave a recovery rate of 33%, which was significantly lower than that obtained by Hachem *et al.* (1995) (96%). The maximum recovery rate was obtained only by using a combination of a selective medium and a nonselective medium in parallel (EYE and

MCHOC). Present data indicate that use of selective and nonselective media in parallel is superior to the use of one medium alone, according to Krajden *et al.* (1987) and Tee *et al.* (1991). We recommend the combined use of EYE and MCHOC for culturing stomach biopsy specimens when it is important to recover living organisms. Because of the material and labor costs involved in producing these media and the fastidious nature of *H. pylori*, which requires prompt processing of biopsy samples, the use of a combination of more than two media is not advisable for laboratories doing routine procedures. This recommendation is particularly relevant considering that two combinations yielded a 100% positivity rate.

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