ISOLATION OF HELICOBACTER PYLORI AFTER TRANSPORT FROM A REGIONAL LABORATORY OF GASTRIC BIOPSY SPECIMENS IN SALINE, PORTAGERM PYLORI OR CULTURED ON CHOCOLATE AGAR

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Summary

Multiple gastric biopsies were taken from 288 patients in Port Lincoln, South Australia. One biopsy was used for a CLOtest and the other three were transported to a central laboratory in Adelaide in physiological saline, Portagerm pylori transport medium or after culture on a chocolate agar plate which was placed in a Biobag. Helicobacter pylori was isolated from 18.3% of patients. There was a 95.7% concordance between culture results and the CLOtest result. Recovery rates after transport on chocolate agar, Portagerm pylori and in saline were 90.2, 90.2 and 84.3%, respectively.

Key words: Helicobacter pylori, transport, Portagerm.

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INTRODUCTION

Culture of Helicobacter pylori from gastric biopsies is one way of diagnosing this infection. More importantly, the determination of antibiotic susceptibilities of individual isolates is likely to become of increasing importance in the management of patients with H. pylori infection. Resistance to metronidazole is well-recognised;1–5 for example, in our population, 60% of isolates are resistant.6 In many centres, 5–15% of isolates are not susceptible to clarithromycin;1–5 resistance may be increasing in our institution, resistance has risen from 3.8% in 1996 to 11.0% in 1999. Resistance or tolerance to amoxycillin has been described recently from Italy and the United States7 and resistance to tetracycline has been observed in 7–11% of patients in Brazil and Nigeria.2,5 Furthermore, in vitro resistance has been correlated with failure to respond to therapy.1,3,8,9

Determination of antibiotic susceptibility requires recovery of the organism following upper gastrointestinal endoscopy. Such endoscopies are often carried out in regional centres where laboratory facilities for the isolation of H. pylori are unavailable. We conducted a trial, therefore, to compare isolation rates when specimens were transported by three different methods from a regional centre, Port Lincoln, to a central laboratory in Adelaide.

MATERIAL AND METHODS

Four antral biopsies were taken from 278 consecutive patients who underwent upper gastrointestinal endoscopy at the Port Lincoln Hospital in South Australia between May 1998 and July 2000. Biopsy 1 was inserted into a jar containing 2.5 ml Portagerm pylori agar (bioMerieux, France). Biopsies 2 and 3 were each placed in 1 ml sterile physiological saline in small sterile containers and sent to the IMVS laboratory in Port Lincoln. Biopsy 3 was then cultured onto a chocolate agar plate and placed in a Biobag environmental chamber type C (Becton-Dickinson, Cockeysville, MD, USA). Biopsy 4 was inoculated into a CLOtest (Bentley, WA) in order to assess urease production; it was incubated at room temperature and read the same day and up to 24 h later according to the manufacturer’s instructions.

The first three biopsies were kept at environmental temperatures and sent by air to Adelaide. They arrived at The Queen Elizabeth Hospital approximately 12 h after the biopsies were taken. The chocolate agar plate was placed in an incubator at 35°C, while the biopsies in saline and Portagerm pylori agar were placed in the refrigerator at 4°C. The specimens were then processed next morning approximately 24 h after they were taken. Methods used for the culture and identification of H. pylori have been described earlier.6 In brief, biopsies were plated onto chocolate agar and incubated at 35°C in CampyPak Plus jars (Becton-Dickinson). Plates were examined after 3 and 6 days. Suspect colonies were Gram-stained and tested for the production of oxidase, catalase and urease. Organisms were identified on the basis of typical colonial and Gram-stain morphology and positivity for oxidase, catalase and urease.

RESULTS

Biopsies were collected from 278 consecutive patients. All specimens from one patient were overgrown with Proteus so this patient was discarded from the analysis. Two further patients had overgrowth of Proteus in one of the three specimens; these patients were retained in the analysis. Helicobacter pylori was not grown in any specimen from 226 patients; it was grown in one or more specimens from 51 patients (18.3%). There was complete concordance between all three transport methods in 38 of these positive patients. The specimens cultured on chocolate agar were positive in 46 of these 51 patients (90.2%) (one specimen was overgrown with Proteus). Likewise, 46 of 51 specimens (90.2%) transported in Portagerm pylori were positive. Of 51 specimens transported in saline, 43 (84.3%) were positive (one specimen was overgrown with Proteus). In the 13 discrepant patients, there was no discernible
pattern, with one or two of any of the transport types being negative (Table 1).

There were only 12 discrepant results when the CLO test result was compared with culture. In nine patients, the CLO test was positive, while culture was negative. The remaining three patients were negative by the CLO test but were positive by culture (one chocolate + Portagerm positive, one chocolate + saline positive, one chocolate only positive).

**DISCUSSION**

There has been a limited number of studies of the effects of transport conditions on the survival of *H. pylori*. Most of these studies report manipulation of transport systems in the laboratory rather than describing field investigations of the transport of specimens. Soltesz et al. 10 examined the effects of temperature and holding times on the recovery of *H. pylori* in Stuart’s transport medium and in physiological saline. Recovery was slightly higher from Stuart’s medium than from saline. Soltesz et al. 10 performed experiments on recovering *H. pylori* from patients who were positive by culture (one chocolate + Portagerm positive, one chocolate + saline positive, one chocolate only positive).

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<th>Chocolate</th>
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+, culture positive; –, culture negative; NA, not available because of overgrowth of Proteus.

Our study examined recovery of *H. pylori* under field conditions reflecting real life. The culture rate of 18% is probably a reasonably accurate reflection of the true prevalence of infection in this patient population. There was concordance between the culture results and the urease test in 95.7% of the patients. In the small number of patients with discrepant results, three-quarters were urease-positive and culture-negative, while the remainder had the reverse findings. Such findings are not uncommon and may reflect sampling error and technical factors, as is evidenced by the variability seen among the three transport methods with respect to culture results. Furthermore, we have previously observed that reading CLO tests at 24 hours is more likely to give a false-positive result than when read at 1 hour. Thus, the CLO-test-positive, culture-negative samples could indicate false urease positivity, culture failure or both. Although reading CLO tests at up to 24 hours may give a number of false-positive reactions, these are the manufacturer’s instructions and are likely to be followed in the real world and so were utilised in this field study.

There were no differences in culture rates between specimens cultured on chocolate agar and transported in a Biobag and those sent in Portagerm pylori, with a single specimen in either system having a 90% chance of success. Specimens transported in saline were almost as effective with *H. pylori* being grown in over 84% of cases. All specimens will need to be cultured onto an agar plate, whether at a regional laboratory or at the central laboratory. Portagerm pylori transport media currently cost $4.13, the Biobag costs $2.18 and saline in a sterile container costs approximately 35 cents. If endoscopies are performed in close proximity to a laboratory with rudimentary microbiological facilities, plating biopsies on chocolate agar and transporting in a Biobag system is likely to be the most efficient and cost-effective system. If such facilities are not available, good results can be expected with specimens being transported in either Portagerm pylori or saline, although some may prefer the former, despite the extra cost, if transport is likely to take more than 12 hours. Transport in saline is likely to be quite acceptable for specimens taken by gastroenterologists working in suburban areas of major cities with transport times of several hours or less.

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**References**