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Recovery of *Helicobacter pylori* from Gastric Biopsy Specimens Is Not Dependent on the Transport Medium Used

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The survival of six clinical isolates of *Helicobacter pylori* at room temperature was investigated after suspension in five different media: brucella broth with 5% lysed horse blood (BBLH), phosphate-buffered saline (PBS), 20% glucose, Stuart medium, and PBS with 10% Fildes enrichment (PBS-F). Only in BBLH and PBS-F no decrease in mean bacterial numbers was observed during the 24-h study period. No *H. pylori* isolates could be cultured from Stuart medium after 7 h of incubation. In contrast, the recovery rates in PBS-F or Stuart medium of *H. pylori* isolates from gastric tissue specimens collected from 19 *H. pylori*-positive patients were not significantly different even after a delay of culture of up to 24 h. Our data show that the medium composition is not critical for the survival of *H. pylori* within gastric tissue specimens.

Helicobacter pylori causes gastritis and peptic ulcer disease. Furthermore, *H. pylori* is highly associated with stomach cancer (7). *H. pylori* infections can effectively be treated with antimicrobial agents, so there is a need for in vitro susceptibility testing. Therefore, among the diagnostic tests available, culturing of *H. pylori* remains important. In addition, this technique will provide *H. pylori* strains for further investigations.

For optimal recovery rates, the viability of *H. pylori* isolates must be maintained during transportation of the gastric biopsy specimens to the microbiological laboratory. Several studies have addressed the influence of transport conditions on the cultivability of *H. pylori* isolates (1–6, 11–14). Some investigators emphasize the need for the rapid transport of biopsy specimens in an appropriate transport medium at low temperature to minimize the loss of bacterial viability (2, 6, 11). Others demonstrate that *H. pylori* can survive at room temperature for 24 h without loss of the ability to recover the organism (13, 14). The main difference between the various studies is the use of either *H. pylori* culture suspensions or *H. pylori*-positive gastric tissue specimens to study the influence of different parameters on viability. Therefore, we tested the survival of six clinical isolates of *H. pylori* as culture suspensions in five potential transport media. Subsequently, the two media showing the most discrepant results with respect to supporting the survival of *H. pylori* isolates were selected for use in the collection of gastric tissue specimens in order to test the recovery rates of *H. pylori* when culture was performed shortly after collection or after a delay of 24 h at room temperature.

Physiological saline suspensions were made from six clinical isolates of *H. pylori* recovered from gastric tissue specimens during routine testing. The bacteria had been stored at −80°C in brain heart infusion with 20% glycerol and were recultured under microaerophilic conditions on blood agar.

In each experiment the survival rates of one clinical isolate of *H. pylori* suspended in each of the following media were investigated: brucella broth (Difco, Detroit, Mich.) supplemented with 5% lysed horse blood (BBLH), phosphate-buffered saline (PBS), 20% glucose (GLUC), Stuart medium (10) (Oxoid, Basingstoke, United Kingdom), and PBS with 10% Fildes enrichment (PBS-F; Oxoid). PBS-F was selected because this medium proved to be excellent for use in the preservation of anaerobic bacteria, suggesting that it provides a protective effect from the deleterious effects of oxygen (8). A total of 100 μl of *H. pylori* isolates from the different suspensions was added to 1 ml of the different media tested to obtain an inoculum of approximately 10^9 CFU/ml. Immediately after inoculation and after 2, 5, 7, and 24 h at room temperature, 200-μl volumes of serial 10-fold dilutions were plated onto blood agar. After 5 days of incubation under microaerophilic conditions, the number of CFU was counted. For each time point the means numbers of CFU per milliliter were calculated from the log-transformed numbers of *H. pylori* colonies obtained for the six clinical isolates in different experiments. Analysis of variance was used to check for differences between means of the log CFU obtained for each medium at the different time points tested as well as between media after a similar period of incubation. The unpaired Student *t* test was used for analysis of differences between two mean values of log-transformed CFU counts.

Thirty-three patients from the upper gastrointestinal endoscopy program were included in the study. Endoscopy was performed with Olympus GIFQ10, Q20, 1T10, and 1T100 endoscopes. Five biopsy specimens from the antrum were taken for culture. One specimen was collected in BBLH on ice and was cultured within 5 h after endoscopy by the standard procedure at our laboratory (10). Two biopsy specimens were put into PBS-F, and another two biopsy specimens were put into Stuart medium. Within 5 h one of the biopsy specimens collected in each of the two media was cultured on Belo Horizonte Agar (9) by rubbing the specimen several times over the agar surface. After a delay of 24 h the other two specimens were cultured. Agar plates were incubated in a humid atmosphere under microaerophilic conditions. After 5 days the plates were checked for the presence of *H. pylori*-like gold-colored colonies. *H. pylori* was further identified by testing for the production of urease, oxidase, and catalase by routine bacteriological techniques. A patient was considered to be positive for *H.*

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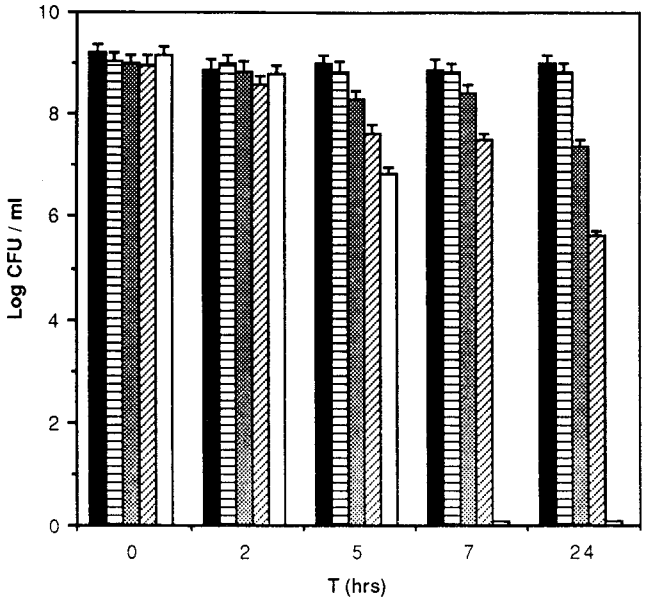

FIG. 1. Recovery of *H. pylori* from bacterial suspensions by using different transport media sampled at various time intervals. ■, BBLH; ⊟, PBS-F; ▨, PBS; ▨, GLUC; □, Stuart medium.

pylori when at least one of the biopsy specimens cultured was positive for *H. pylori*. The Fisher test was used for analysis of differences between recovery rates from gastric biopsy specimens transported in Stuart medium or PBS-F.

When testing culture suspensions of *H. pylori* no significant decrease in the mean numbers of bacteria was found during incubation for 24 h at room temperature in BBLH or PBS-F, whereas those in PBS, Stuart medium, and GLUC declined significantly during the study period (Fig. 1). The mean numbers of CFU of *H. pylori* did not decrease during the first 2 h of incubation in any of the media tested ($P > 0.05$). At 5 h significantly lower numbers of *H. pylori* isolates were recovered, in declining order, from PBS, Stuart medium, and GLUC, compared with the numbers detected in BBLH or PBS-F. At 7 h viable counts were further reduced in GLUC ($P < 0.05$), and no *H. pylori* was recovered from Stuart medium. This is in agreement with the results obtained by others (6, 11). After 24 h of incubation the mean numbers of *H. pylori* in BBLH and PBS-F were not reduced compared with the numbers in the initial inoculum ($P > 0.05$). In successive order, significantly decreased mean viable counts were found in PBS, GLUC, and finally, Stuart medium, from which no *H. pylori* could be detected. To test whether the survival of *H. pylori* was dependent on the inoculum size used, *H. pylori* was suspended in PBS-F to mean numbers of 4.1×10^6 and 4.2×10^3 CFU/ml. No changes in viable counts were observed during the observation period of 24 h ($P > 0.05$) (data not shown).

Our data show that for several transport media, low temperature is not necessary for substantial survival of *H. pylori* in culture suspensions. This is in agreement with data from Xia et al. (14), who demonstrated that the recovery of *H. pylori* was 100% when the organism was suspended to 10^6 CFU/ml in brain heart infusion with 10% horse serum and storage at room temperature for 24 h. Even after 3 days, 10 of 12 strains tested were recovered. However, they did not measure the survival of *H. pylori* quantitatively.

When testing gastric tissue specimens collected in PBS-F and Stuart medium, the rates of recovery of *H. pylori* were not significantly different for both media. Gastric tissue specimens from 19 *H. pylori*-positive patients collected in BBLH on ice or PBS-F and cultured within 5 h were all positive for *H. pylori*, as were 18 of 19 biopsy specimens collected in Stuart medium (one overgrown) ($P > 0.05$). When culture was delayed for 24 h, 18 of 19 biopsy specimens collected in PBS-F were *H. pylori* culture positive, none was negative, and 1 was overgrown, whereas at 24 h, 16 of 19 biopsy specimens collected in Stuart medium were *H. pylori* culture positive, 2 were negative, and 1 was overgrown ($P > 0.05$).

Generally, it is emphasized that transport at a relatively low temperature is essential for obtaining a satisfactory recovery of *H. pylori* from gastric tissue specimens. In Stuart medium, recovery rates of up to 100% after 24 h were found when gastric specimens were kept at 4°C (5), which corresponds to the data obtained by studying the survival of *H. pylori* in suspensions at a low temperature in the same medium (10). On the other hand, discrepancies were observed with respect to the survival of *H. pylori* in physiological saline. Whereas physiological saline supported the viability of *H. pylori* in suspension very poorly (3, 11), in another study a recovery rate of 100% was found after a delay of culture of gastric biopsy specimens for 24 h at a similar temperature (13). A similar observation was made in our study. Despite the complete loss of viability of clinical *H. pylori* isolates suspended in Stuart medium after 24 h, the recovery rate of *H. pylori* from biopsy specimens transported in this medium and cultured after a delay of 24 h was not statistically different from that from biopsy specimens transported in PBS-F, which supports the viability of *H. pylori* in suspension very well. The major function of a transport medium may therefore be to prevent the gastric tissue specimen from drying.

It can be concluded that the survival of *H. pylori* in suspension at room temperature is highly dependent on the medium used. In contrast, the composition of the medium for transportation of gastric biopsy specimens was not critical for the recovery of *H. pylori*, even when culture was delayed for 24 h at room temperature. Therefore, gastric biopsy specimens can be transported from the endoscopy room to the microbiological laboratory at room temperature when specimens are cultured for the presence of *H. pylori* within 24 after collection. Because bacterial overgrowth was observed in a few cases, physiological saline, although not tested in the present study, might be preferred because of the lack of nutrients for bacterial growth.

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