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***Basic Study***

**Combined antrum and corpus biopsy protocol improves *Helicobacter pylori* culture success**

Brennan DE *et al*. Improving *H. pylori* culture success

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**Abstract**

BACKGROUND

*Helicobacter pylori* (*H. pylori*) causes chronic gastritis, peptic ulcer disease, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma. Eradication rates have fallen, mainly due to antimicrobial resistance. Consensus guidelines recommend that first-line treatment is based on the local prevalence of antimicrobial resistance and that rescue therapies are guided by antimicrobial susceptibility testing (AST). However, *H. pylori* culture is challenging and culture-based AST is not routinely performed in the majority of hospitals. Optimisation of *H. pylori* culture from clinical specimens will enable more widespread AST to determine the most appropriate antimicrobials for *H. pylori* eradication.

AIM

To determine whether dual antrum and corpus biopsy sampling is superior to single antrum biopsy sampling for *H. pylori* culture.

METHODS

The study received ethical approval from the joint research ethics committee of Tallaght University Hospital and St. James’s Hospital. Patients referred for upper gastrointestinal endoscopy were invited to participate. Biopsies were collected in tubes containing Dent’s transport medium and patient demographics were recorded. Biopsies were used to inoculate Colombia blood agar plates. Plates were incubated under microaerobic conditions and evaluated for the presence of *H. pylori.* Statistical analyses were performed using Graphpad PRISM. Continuous variables were compared using the two-tailed independent *t*-test. Categorical variables were compared using the two-tailed Fisher exact test. In all cases, a *P* value less than 0.05 was considered significant.

RESULTS

In all, samples from 219 *H. pylori*-infected patients were analysed in the study. The mean age of recruited patients was 48 ± 14.9 years and 50.7% (*n* = 111) were male. The most common endoscopic finding was gastritis (58.9%; *n* = 129). Gastric ulcer was diagnosed in 4.6% (*n* = 10) of patients, while duodenal ulcer was diagnosed in 2.7% (*n* = 6). Single antrum biopsies were collected from 73 patients, whereas combined antrum and corpus biopsies were collected from 146 patients. There was no significant difference in age, sex or endoscopic findings between the two groups. *H. pylori* was successfully cultured in a significantly higher number of cases when combined antrum and corpus biopsies were used compared to a single antrum biopsy [64.4% (*n* = 94/146) *vs* 49.3% (36/73); *P* = 0.04)].

CONCLUSION

Combined corpus and antrum biopsy sampling improves *H. pylori* culture success compared to single antrum biopsy sampling.

**Key Words:** *Helicobacter pylori*; Culture; Antimicrobial susceptibility testing; Antimicrobial; Antrum; Corpus

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**Core Tip:** *Helicobacter pylori (H. pylori*) antimicrobial susceptibility testing is critical to accurately detect antimicrobial resistance, thereby influencing appropriate treatment choices, promoting antimicrobial stewardship and increasing *H. pylori* eradication rates. However, *H. pylori* culture represents a challenge and is limited to a small number of specialized centres and reference laboratories. Increasing biopsy sample number has been suggested to improve culture success, but data directly comparing dual biopsy *vs* single biopsy sample collection for *H. pylori* culture are lacking. Here we show that combined corpus and antrum biopsy sampling improves *H. pylori* culture success compared to single antrum biopsy sampling.

**INTRODUCTION**

*Helicobacter pylori* (*H. pylori*)causes one of the most common bacterial infections globally, colonising the stomach of approximately half of the world’s population.This bacteriumis of interest clinically as the causative agent of chronic gastritis, peptic ulcer disease, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma. *H. pylori* has been designated a class I carcinogen by the World Health Organisation (WHO)[1]. Treatment usually involves stomach acid suppression using a proton pump inhibitor (PPI) together with 2-3 antimicrobials. However, treatment success has been impacted in recent years, largely due to the emergence of antimicrobial-resistant *H. pylori*. Indeed, the WHO has included *H. pylori* on their priority list of antibiotic-resistant microorganisms[2].

Primary resistance rates for clarithromycin, metronidazole and levofloxacin are 15% or higher in nearly all WHO regions[3]. Recent data on *H. pylori* antimicrobial resistance in European countries revealed overall primary resistance rates of 21.4%, 15.8% and 38.9% for clarithromycin, levofloxacin and metronidazole, respectively[4]. As resistance rates vary from region to region[3-5], consensus guidelines[6-11] recommend that first-line treatment for *H. pylori* is based on primary resistance rates in a given population. If the prevalence of primary clarithromycin resistance is unknown, it is recommended to perform clarithromycin antimicrobial susceptibility testing (AST) before using clarithromycin-based first-line triple therapy. *H. pylori* AST is also recommended to guide rescue therapy following 2 treatment failures[8]. Thus, methods to detect antimicrobial resistance are of great importance both for surveying resistance rates in different regions and for personalising *H. pylori* treatment. Traditionally, *H. pylori* AST has been performed by culturing the bacteria from stomach tissue biopsies taken during endoscopic examination and determining the minimum inhibitory concentration of an antimicrobial agent required to inhibit bacterial growth[12]. But *H. pylori* is a fastidious organism and culture is challenging and time-consuming with reported success rates varying from 55%-93%[13,14]. Culture success is influenced by many factors, including PPI use, tissue sampling site, choice of transport medium and *H. pylori* growth conditions[4,15]. This study aimed to determine whether a dual antrum and corpus biopsy sampling protocol was superior to a single antrum biopsy protocol for the successful culture of *H. pylori*.

**MATERIALS AND METHODS**

***Study design and ethics***

The study was carried out at Tallaght University Hospital, Dublin, Ireland, which is affiliated with Trinity College Dublin. The study received ethical approval from the joint research ethics committee of Tallaght University Hospital and St. James’s Hospital. Patients referred for upper gastrointestinal endoscopy were invited to participate. Patients were prospectively recruited to determine the culture success rate when combined antrum and corpus biopsies were used. The culture success rate when single antrum biopsies were used was determined retrospectively.

***Study population***

Inclusion criteria were (1) Ability and willingness to participate in the study and to provide informed consent; and (2) Confirmed *H. pylori* infection as indicated by a positive rapid urease test (TRI-MED Distributors, PTY LTD, Washington, United States) at 30 min and by histology. Exclusion criteria were (1) Age less than 18 years; (2) Pregnancy or lactation; (3) Severe intercurrent illness; (4) Recent antimicrobial use (within 4 wk); and (5) Bleeding problems or use of blood thinning drugs.

***Sample collection***

At endoscopy, biopsy samples from each patient were placed directly into collection tubes containing Dent’s transport medium [brain heart infusion broth containing 2.5% (w/v) yeast extract, 5% sterile horse serum and *H. pylori* Selective Supplement (Oxoid, Basingstoke, United Kingdom)]. When both antrum and corpus biopsies were collected from a patient, the two tissue samples were placed into the same collection tube. Biopsy samples were processed for culture as soon as possible following endoscopy, usually within 6 h. If processing was delayed, samples were refrigerated at 4 °C and used to inoculate plates within 24 h.

***H. pylori culture***

The tissue samples were inoculated onto Columbia blood agar plates containing 5% laked horse blood (VWR International, Lutterworth, Leicestershire, United Kingdom) and incubated at 37 °C under microaerobic conditions generated using the CampyGen 2.5 L Atmosphere Generation System (Oxoid). When both antrum and corpus biopsies were collected, they were inoculated onto the same plate. Plates were examined for the presence of *H. pylori* for up to 7 d. *H. pylori* was identified by visual inspection of the colonies, a positive urease test and by polymerase chain reaction.

***Statistical analysis***

Statistical analysis was performed using GraphPad Prism (GraphPad Software Inc., CA, United States). Continuous variables are presented as arithmetic mean and standard deviation. Continuous variables were compared using the two-tailed independent *t*-test. Categorical variables are presented as percentages and their 95% confidence intervals (95%CI). Categorical variables were compared using the two-tailed Fisher exact test. In all cases, a *P* value less than 0.05 was considered significant.

**RESULTS**

In all, samples from 219 *H. pylori*-infected patients were analysed. The mean age of recruited patients was 48 ± 14.9 years and 50.7% were male (Table 1). The most common endoscopic finding was gastritis (58.9%; *n* = 129). The rates of more serious *H. pylori*-associated diseases, such as gastric ulcer, duodenal ulcer and intestinal metaplasia were low in the study cohort at 4.6% (*n* = 10), 2.7% (*n* = 6) and 0.5% (*n* = 1), respectively (Table 1).

Single antrum biopsies were collected from 73 patients, whereas combined antrum and corpus biopsies were collected from 146 patients. There was no significant difference in age, sex or endoscopic findings between the two groups (Table 1). *H. pylori* was successfully cultured in a significantly higher number of cases when combined antrum and corpus biopsies were used compared to a single antrum biopsy [64.4% (*n* = 94/146) *vs* 49.3% (36/73); *P* = 0.04) (Table 2)].

**DISCUSSION**

*H. pylori* AST is critical to accurately detect antimicrobial resistance, thereby influencing appropriate treatment choices, promoting antimicrobial stewardship and increasing *H. pylori* eradication rates. While molecular AST methods are available, these are primarily limited to the detection of clarithromycin-and levofloxacin-associated DNA mutations. Culture-based AST remains the only method currently available to test all the antimicrobials potentially useful for *H. pylori* treatment[16]. Despite the importance of culture-based AST , *H. pylori* culture is not routinely performed in the majority of hospitals[5-7,11] either to survey resistance rates or to tailor therapies. From a microbiology perspective, *H. pylori* is challenging to culture. In this study, we report an increased culture success rate when a dual antrum and corpus biopsy protocol was used compared to using a single antrum biopsy (64.4% *vs* 49.3%; *P* = 0.04). While a significant improvement in culture success was observed, a rate of 64.4% is lower than some previous reports. PPI use is known to impact the diagnostic accuracy of *H. pylori* culture[8]. While patients attending for endoscopy at our centre are encouraged to refrain from PPI use 2 wk prior to their scheduled endoscopy, in practice many do not. Nonetheless, the 15% increase in culture success rate reported here provides a strong rationale for a combined biopsy approach.

It is not surprising that the more biopsy specimens used for culture, the higher the chance of recovering *H. pylori* and this practice has been suggested elsewhere[15,17]. However, recent guidelines on the management of *H. pylori*[6-8,11]do not include specific recommendations on biopsy sampling protocols for *H. pylori* culture and studies directly evaluating culture success using a single *vs* combined biopsy sampling protocol are lacking. The biopsy sampling location is important for a number of reasons. Firstly, collecting biopsies from both the antrum and the corpus takes into account patchy distribution of *H. pylori* in the stomach, which can occur with PPI use[15,18,19]. Furthermore, intragastric location-specific differences in the evolution of *H. pylori* have been reported across strains within the same individual[20]. In terms of AST, it is important to collect biopsies from both sites, as these differences extend to the antimicrobial susceptibility profiles between strains isolated from the corpus and those from the antrum of the same patient[21,22]. Thus, resistance to a given antimicrobial could be missed if biopsy samples from only one location are taken, potentially having a negative impact on treatment outcome.

A limitation of our study is that patients were recruited prospectively to the dual biopsy sampling group, while the single antrum biopsy culture success rate was analysed retrospectively. However, it should be noted that for the entire duration of the patient recruitment and sample collection phases of the study, we followed the standardized protocols of the European *H. pylori* Antimicrobial Susceptibility Testing Working Group[4]. Therefore, the sample transport protocols, microbiological media and culture conditions and methods were consistent throughout the entirety of the study, thereby limiting heterogeneity in this regard.

**CONCLUSION**

In conclusion, combined corpus and antrum biopsy sampling improves *H. pylori* culture success compared to single antrum biopsy sampling.

**ARTICLE HIGHLIGHTS**

***Research background***

*Helicobacter pylori (H. pylori)* represents a public health issue as the causative agent of chronic gastritis, peptic ulcer disease, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma. Success rates for current therapies have fallen over the years, mainly due to antimicrobial resistance. International guidelines recommend that treatment choices are based on local antimicrobial resistance rates. However, *H. pylori* culture is challenging and culture-based antimicrobial susceptibility testing (AST) is not routinely performed in most healthcare facilities.

***Research motivation***

Optimisation of *H. pylori* culture from clinical specimens will enable more widespread AST for *H. pylori*.

***Research objectives***

This research aimed to evaluate biopsy sampling protocols to enhance *H. pylori* culture success, specifically to determine whether dual antrum and corpus biopsy sampling was superior to a single antrum biopsy sampling protocol.

***Research methods***

Stomach tissue biopsies from rapid-urease test positive patients were collected in tubes containing Dent’s transport medium. Biopsies were used to inoculate Colombia blood agar plates. Plates were incubated under microaerobic conditions and evaluated for the presence of *H. pylori.* Culture success rates when a single antrum biopsy was used were compared to those when dual antrum and corpus biopsies were used.

***Research results***

*H. pylori* was successfully cultured in a significantly higher number of cases when combined antrum and corpus biopsies were used compared to a single antrum biopsy sample.

***Research conclusions***

A combined corpus and antrum biopsy sampling approach improves *H. pylori* culture success compared to a single antrum biopsy sampling protocol.

***Research perspectives***

Optimisation of *H. pylori* culture methods will encourage more widespread AST. Antimicrobial resistance surveillance is the key to determining the most appropriate antimicrobials for *H. pylori* eradication.

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**Footnotes**

**Institutional review board statement:** The study was reviewed and approved by the Joint Research Ethics Committee of St. James’s Hospital and Tallaght University Hospital.

**Conflict-of-interest statement:** All authors have nothing to disclose.

**Data sharing statement:** No additional data are available.

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T**able 1 Patient demographics**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Total, *n* = 219** | **Single, *n* = 73**  | **Combined, *n* = 146** | ***P* value1** |
| Mean age (yr) | 48 ± 14.9 | 49 ± 15.9 | 48 ± 14.5 | 0.43 |
| **Sex** |  |  |  | 0.32 |
| Male | *n* = 111 (50.7%) | *n* = 41 (56.2%) | *n* = 70 (47.9%) |  |
| Female | *n* = 108 (49.3%) | *n* = 32 (43.8%) | *n* = 76 (52.1%) |  |
| **Endoscopy findings** |  |  |  |  |
| Normal | 18 (8.2%) | 5 (6.8%) | 13 (8.9%) | 0.80 |
| Gastritis | 129 (58.9%) | 40 (54.8%) | 89 (61.0%) | 0.57 |
| Gastric ulcer | 10 (4.6%) | 3 (4.1%) | 7 (4.8%) | 1.00 |
| Duodenal ulcer | 6 (2.7%) | 2 (2.7%) | 4 (2.7%) | 1.00 |
| Intestinal metaplasia | 1 (0.5%) | 1 (1.4%) | 0 (0%) | 0.33 |
| Duodenitis | 11 (5.0%) | 3 (4.1%) | 8 (5.5%) | 0.76 |
| Oesophagitis | 4 (1.8%) | 3 (4.1%) | 1 (0.7%) | 0.12 |
| Barrett’s oesophagus | 5 (2.3%) | 3 (4.1%) | 2 (1.4%) | 0.34 |
| Hiatus hernia | 9 (4.1%) | 3 (4.1%) | 6 (4.1%) | 1.00 |
| Telangiectasia | 1 (0.5%) | 0 (0%) | 1 (0.7%) | 1.00 |
| Portal hypertensive gastropathy | 1 (0.5%) | 1 (1.4%) | 0 (0%) | 0.33 |
| No data | 24 (11.0%) | 9 (12.3%) | 15 (10.3%) | 0.65 |

1Single versus combined.

Table 2 Culture success rate of *Helicobacter pylori* using single antrum biopsies versus combined antrum and corpus biopsies

|  |  |  |
| --- | --- | --- |
|  | **Culture positivity rate** | ***P* value** |
| Single biopsy  | 49.3% (36/73; 95%CI: 38.2-60.5) | 0.04a |
| Combined biopsies  | 64.4% (94/146; 95%CI: 56.3-71.7) |  |

a*P* value < 0.05.