Technical methods

Evaluation of “CLO-test” to detect Campylobacter pyloridis in gastric mucosa

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Campylobacter pyloridis is strongly associated with the presence of histological gastritis,1 2 but its role in the aetiology of peptic ulcer disease and non-ulcer dyspepsia has yet to be determined. Its detection in antral mucosal biopsy specimens usually entails histological or microbiological methods that may require several days for a result. In a previous study3 we found that direct examination of biopsy specimens by phase contrast microscopy was a rapid and reliable screening method. It has the disadvantage, however, that the necessary equipment is not always readily available. As C pyloridis produces abundant urease4 5 this fact has been used for the rapid detection of its presence in human gastric antral biopsy specimens.6 7 We prospectively evaluated a commercial urease test, CLO-test (Delta West Ltd, Canning Vale, Western Australia 6155) by comparing it with microscopy and culture for C pyloridis in gastric antral mucosa.

Patients and methods

Gastric biopsy specimens were taken from 80 consecutive patients undergoing routine upper gastrointestinal endoscopy. Nineteen of these patients were taking part in two other ongoing trials. Three endoscopic biopsy specimens were obtained from the gastric antral mucosa within a 5 cm radius of the pylorus. One specimen was immediately inserted in the CLO-test, which was held at room temperature for up to 24 hours. It was examined frequently for any colour change from yellow (negative) to orange or bright pink (positive). The two other biopsy specimens were collected in 0·25 ml sterile saline (0·85%) for transport to the laboratory and were processed within three hours. Phase contrast microscopy, Gram stain, and culture were performed as previously described.3 The Gram stain was done retrospectively if only one other test was positive for C pyloridis. The number of Campylobacter like organisms seen on microscopy was scored from + to ++ +.

Isolates were considered to be C pyloridis if they grew as 0.5–1 mm translucent greyish colonies after three days on chocolate (horse) blood agar, and were Gram negative, curved, or S shaped rods which were positive for oxidase, catalase, and rapid urease (Oxoid CM71). Growth occurred under microaerophilic conditions, but not in air.

Results

Eighty antral biopsy specimens were examined by phase contrast microscopy, culture, and CLO-test. Gram stain was also done on 10 specimens when only one of the other tests was positive. The rapid urease test was positive for 47 specimens, two of which were orange at 24 hours rather than the characteristic pink produced by the other C pyloridis positive specimens. C pyloridis was detected in 45 of these by both microscopy and culture, and in one by microscopy alone. For one specimen, the rapid urease test was an orange colour after 24 hours, but C pyloridis was not otherwise shown. C pyloridis was isolated from a further five specimens which gave a negative urease result. The other 28 specimens were negative by phase contrast microscopy, culture, and CLO-test (table 1).

Thus with the final reading at 24 hours, CLO-test had a sensitivity of 90% (five false negative CLO-tests from 51 microscopically or culture positive, or both, specimens) and a specificity of 97% (one false positive CLO-test from 29 negative specimens).

Of the 47 specimens that were CLO-test positive, 31 were positive within 30 minutes, a further six were positive by one hour, while the rest were positive after two hours or more (table 2). In addition, there was a positive correlation (p < 0.001) between the number of typical curved bacteria seen on phase contrast microscopy and the time required for CLO-test to become positive (figure). This correlation was restricted to those 41 specimens which were positive by two hours.

Discussion

Using the commercially available CLO-test, this study confirmed previous reports6 7 8 that testing for urease is a rapid and reliable method for showing the presence of C pyloridis in gastric antral biopsy specimens; and furthermore, with 79% of positive tests occurring within one hour, these results closely paral-
Table 1 Comparison of detection methods for *C pyloridis*

<table>
<thead>
<tr>
<th>No of antral biopsy specimens</th>
<th>CLO-test</th>
<th>Phase contrast microscopy</th>
<th>Culture</th>
<th>Gram stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ *C pyloridis* detected; – *C pyloridis* not detected; ND = not done.

Table 2 Reaction time for positive CLO-test

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>No of antral biopsy specimens (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>31 (66)</td>
</tr>
<tr>
<td>1</td>
<td>37 (79)</td>
</tr>
<tr>
<td>2</td>
<td>41 (87)</td>
</tr>
<tr>
<td>24</td>
<td>47 (100)</td>
</tr>
</tbody>
</table>

equipment and the incorporation of an antimicrobial agent in the urease test, such as is present in the CLO-test should minimise possible false positive results due to these bacteria.

While the CLO-test is reliable in the initial screening of patients who first present with upper gastrointestinal symptoms, further studies are needed to determine the reliability of a negative test in patients being re-evaluated after treatment. In this study, of the five patients with a false negative CLO-test, two had been treated with an antiulcer agent.

In contrast to the findings of our previous study, phase contrast microscopy had a lower detection rate than culture for *C pyloridis*. Of those eight patients who were negative by phase contrast microscopy but culture positive, three had recently been receiving antiulcer treatment, and one had had a parietal cell vagotomy. It is probable that bacteria damaged by drugs are not readily recognised by this microscopic method. These results suggest that during therapeutic trials, a negative CLO-test may need to be confirmed by culture for *C pyloridis*.

References


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Evaluation of "CLO-test" to detect Campylobacter pyloridis in gastric mucosa.
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doi: 10.1136/jcp.40.4.462

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