Urease-positive bacteria in the stomach induce a false-positive reaction in a urea breath test for diagnosis of *Helicobacter pylori* infection

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INTRODUCTION

Invasive and non-invasive diagnostic methods are used to detect *Helicobacter pylori* infection. Invasive methods using endoscopically obtained gastric biopsy specimens primarily include histopathological examination, a rapid urease test (RUT) and isolation of *H. pylori* by culture. Non-invasive methods include an anti-*H. pylori* antibody test, an [*13C*]urea breath test (UBT) and a stool antigen test (Kusters et al., 2006). In patients treated with proton pump inhibitors and antibiotics for the eradication of *H. pylori*, urease-producing bacteria other than *H. pylori* were isolated and identified from the oral cavity and stomach. In 4/102 patients, the UBT gave false-positive results. These false-positive results were found to be caused by the presence of urease-positive bacteria in the oral cavity and stomach. Five bacterial species with urease activity (*Proteus mirabilis*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Staphylococcus aureus*) were subsequently isolated from the oral cavity and/or stomach. As there was no correlation between the *in vitro* urease activity of urease-positive non-*H. pylori* bacteria and the UBT value, and all of the patients with a false-positive UBT result were suffering from atrophic gastritis, it is possible that the false-positive results in the UBT were a result of colonization of urease-positive bacteria and gastric hypochlorhydric conditions. Thus, for the diagnosis of *H. pylori* infection using a UBT, the influence of stomach bacteria must be considered when interpreting the results.

This study investigated the influence of urease-positive non-*Helicobacter pylori* bacteria on the results of a urea breath test (UBT) to evaluate the diagnostic utility of a UBT using film-coated [*13C*]urea tablets. The UBT was performed in 102 patients treated with a proton pump inhibitor and antibiotics for the eradication of *H. pylori*. Urease-producing bacteria other than *H. pylori* were isolated and identified from the oral cavity and stomach. In 4/102 patients, the UBT gave false-positive results. These false-positive results were found to be caused by the presence of urease-positive bacteria in the oral cavity and stomach. Five bacterial species with urease activity (*Proteus mirabilis*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Staphylococcus aureus*) were subsequently isolated from the oral cavity and/or stomach. As there was no correlation between the *in vitro* urease activity of urease-positive non-*H. pylori* bacteria and the UBT value, and all of the patients with a false-positive UBT result were suffering from atrophic gastritis, it is possible that the false-positive results in the UBT were a result of colonization of urease-positive bacteria and gastric hypochlorhydric conditions. Thus, for the diagnosis of *H. pylori* infection using a UBT, the influence of stomach bacteria must be considered when interpreting the results.

**METHODS**

**Patients.** Between April 2003 and March 2004, 102 patients (83 men and 19 women; aged 14–80 years) at Yamagata Prefectural Central Hospital, Japan, who were diagnosed as having peptic ulcer diseases with *H. pylori* infection were analysed. These patients were recruited randomly and sequentially to form a cohort of at least 100 cases. Patients were treated with 60 mg lansoprazole or 40 mg omeprazole daily and two antibiotics (400 or 800 mg clarithromycin and 1500 mg amoxicillin) for 1 week to eradicate *H. pylori* at Yamagata Prefectural Central Hospital.

**Evaluation of *H. pylori* status.** From 4 to 6 weeks after the end of the eradication therapy, patients were evaluated for *H. pylori* status by UBT using film-coated [*13C*]urea tablets (UBIT; Otsuka

Abbreviations: RUT, rapid urease test; UBT, urea breath test.
Table 1. H. pylori status in patients treated by eradication therapy

A total of 102 cases (83 males, 19 females; mean age 54.6 ± 12.8 years; range 19–80 years) were examined.

<table>
<thead>
<tr>
<th>UBT*</th>
<th>RUT</th>
<th>Culture</th>
<th>No. of cases (%)</th>
<th>H. pylori status</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td></td>
<td></td>
<td>4 (3.9)</td>
<td>Negative†</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td></td>
<td>1 (1.0)</td>
<td>Positive</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td></td>
<td>7 (6.9)</td>
<td>Positive</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td></td>
<td>90 (88.2)</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*The cut-off value for the UBT was 2.5% at 20 min.
†Four cases that were UBT-positive, culture-negative and RUT-negative in two sequential gastroendoscopic examination tests were judged to be false-positive UBT results.

For identification of Gram-positive and catalase-positive cocci. All urease-positive bacteria were stored at −30 °C until further testing.

Quantification of the in vitro urease activity of urease-producing bacterial isolates. For measurement of in vitro urease activity, samples and urea/phenol red solution (pH 7.4) were incubated at 37 °C. Colour change was measured at A595 at each time point. Based on a standard curve obtained from an experiment using urease from jack beans (Wako Chemical), the urease titre was determined as U (mg protein)−1.

The urease activity of urease-producing bacteria, including H. pylori, was quantified at various pH values to examine the effect of pH on urease activity. The rate of urea conversion into ammonia was measured by mixing a urea solution (final concentration 250 mM) with urease-producing bacteria suspended in 0.1 M citrate buffer with the pH adjusted to 1, 3 or 5 by the addition of HCl. The amount of ammonia produced was quantified using an ammonia test (Wako Chemical), as described previously (Kamiya et al., 1993). As a positive control in the urease assay, H. pylori strains TK1029 and TK1402 were used.

RESULTS AND DISCUSSION

Evaluation of H. pylori status in patients undergoing eradication therapy

All 102 patients completed the eradication protocol. Based on the results of the UBT using film-coated [13C]urea tablets, 90 patients with low UBT values (<2.5%) were considered H. pylori-negative and seven patients with a high UBT value (10% or higher) that were RUT-positive and culture-positive were considered H. pylori-positive (Table 1). Five patients showed borderline results in the UBT. As one of these five patients was RUT-positive, this patient was diagnosed as H. pylori-positive. The remaining four patients were RUT-negative and culture-negative in the second tests, so were considered to be H. pylori-negative and cytochrome oxidase-negative rods and API Staph strips for identification of Gram-positive and catalase-positive cocci. All urease-positive bacteria were stored at −30 °C until further testing.
negative (3/4 patients showed borderline results in the second UBT). The final eradication rate was 92.2% (94/102 patients). The four cases (all male) that were UBT-positive, culture-negative and RUT-negative on two sequential gastroendoscopic examinations were judged to be false positives in the UBT. In these four patients, UBT values at 20 min were significantly higher than those at 5 min, suggesting the influence of urease-producing bacteria in the stomach rather than in the oral cavity. Three patients (Table 2; cases 1, 2 and 4) with a false-positive UBT result were aged over 70 years and suffered from atrophic gastritis (all cases), gastric ulcer (cases 1 and 4) and gastric cancer (case 2). These patients also had prostatic hypertrophy. Two patients (cases 2 and 3) were smokers and one patient (case 2) had high levels of alcohol usage. The remaining patient (Table 2; case 3) was aged 41 and suffered from duodenal stenosis with ulcer scar formation. The mean age (66.3 ± 16.9 years old) of the false-positive patients was higher than that of all the patients (54.6 ± 12.8 years old). There was no correlation between smoking habit, alcohol usage, medication and UBT results (data not shown).

Thus the UBT is a highly accurate test, with a sensitivity and specificity of more than 90% (Lerang et al., 1998; Savarino et al., 1999; Gomez et al., 2002; Perri et al. 2002). Ohara et al. (2004) reported that a UBT using film-coated [13C]urea tablets resulted in better accuracy compared with a standard UBT in Japanese patients. The present study confirms that a UBT using film-coated [13C]urea tablets has a low false-positivity rate (3.9%: 4/102 patients) among patients in whom eradication therapy was indicated. In a clinical trial evaluating a standard UBT using the same protocol as the present study, we found the false-positivity rate to be 7.5% (22/294 cases; data not shown), which is significantly higher than the false-positivity rate in the UBT using film-coated [13C]urea tablets. However, even when a film-coated, tablet-based UBT is used, false-positive results are seen in some patients, particularly in those with suppression of gastric acid secretion, such as elderly patients over 70 years old with atrophic gastritis after eradication therapy.

### Isolation of urease-producing bacteria from stomach biopsy specimens and throat swabs

In the UBT false-positive cases, urease-producing bacteria were isolated from the stomach and/or oral cavity. The isolates were identified as Gram-negative Proteus mirabilis, Citrobacter freundii, Klebsiella pneumoniae and Enterobacter cloacae and Gram-positive Staphylococcus aureus (Table 2). In three out of four cases, members of the Enterobacteriaceae were detected. In one false-positive case (case 4), a very high UBT value (152.2% positives in the UBT. In these four patients, UBT values at 20 min were significantly higher than that of all the patients (54.6 ± 12.8 years old). There was no correlation between smoking habit, alcohol usage, medication and UBT results (data not shown).

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<table>
<thead>
<tr>
<th>Case</th>
<th>First test</th>
<th></th>
<th>Isolates from oral cavity*</th>
<th></th>
<th>Second test</th>
<th></th>
<th>Isolates from oral cavity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.1</td>
<td>3.1</td>
<td>P. mirabilis</td>
<td>P. mirabilis</td>
<td>54</td>
<td>14.6</td>
<td>P. mirabilis</td>
</tr>
<tr>
<td>2</td>
<td>0.7</td>
<td>2.9</td>
<td>ND</td>
<td>K. pneumoniae</td>
<td>0.6</td>
<td>0</td>
<td>K. pneumoniae</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>4.2</td>
<td>K. pneumoniae</td>
<td>ND</td>
<td>2.2</td>
<td>4.5</td>
<td>S. aureus</td>
</tr>
<tr>
<td>4†</td>
<td>1.8</td>
<td>5.9</td>
<td>ND</td>
<td>ND</td>
<td>0.3</td>
<td>152.2</td>
<td>ND</td>
</tr>
</tbody>
</table>

- ND, None detected.
- *The oral urease-positive non-H. pylori bacteria were isolated from swabs obtained from within the oral cavity.
- †For the case 4 patient, a third UBT was performed, which gave a value of 0.3% at 20 min.
gastroschisis at birth and showing decreased gastric movement, urease-positive Micrococcus in the gastric mucosa induced a false-positive result in a UBT. Brandi et al. (2006) recently reported that ten strains of urease-positive non-H. pylori bacteria (five genera: Streptococcus, Staphylococcus, Gardnerella, Lactococcus and Enterococcus), among which Staphylococcus capitis subsp. urealyticus showed the strongest urease activity, were isolated from the stomachs of six hypochlorhydric patients. These urease-positive non-H. pylori bacteria were thought to be responsible for false-positive results in the UBT. Gurbuz et al. (2005) detected yeast-like micro-organisms in the gastric mucosa of all patients with false-positive results in a UBT. A higher proportion of false-positive results (24%: 8/34 cases) in the UBT may be due to the presence of the yeast-like micro-organisms, although in vitro urease activity was not examined in that study.

A total of 59 and 12 urease-producing bacterial strains were isolated from negative and false-positive UBT cases, respectively. The in vitro urease activity of 39 and 8 urease-positive bacteria isolated from negative and false-positive UBT cases, respectively, was quantified (Table 3). There were no significant differences in urease activity between UBT-negative and UBT-false-positive case-derived isolates. A total of 20 and 51 urease-producing bacterial strains were isolated from gastric biopsy specimens and throat swabs, respectively. When the urease activity of urease-producing bacteria isolated from stomach biopsies (n=10) and throat swabs (n=37) was compared, no significant differences were observed between false-positive and negative cases (Fig. 1). Correlations between UBT values and in vitro urease activities of urease-positive bacteria other than H. pylori isolated from the stomach and oral cavity were then examined. No significant correlations between in vitro urease activity and UBT values were observed (Fig. 2).

Effects of pH on urease activity of isolates

In order to examine the effects of pH on the urease activity of urease-producing bacteria (except for E. cloacae isolated from false-positive UBT case 2), ammonia production was measured at pH values of 1, 3 and 5 (Table 4). The urease activity of H. pylori was found to have a wide optimal pH range. Among other urease-producing bacteria, the urease activity of P. mirabilis was highest, followed by that of S. aureus. Unlike H. pylori, the urease activity of these bacteria was markedly inhibited under acidic conditions (pH 1).

In the present study, 12 strains from five species (P. mirabilis, C. freundii, K. pneumoniae, E. cloacae and S. aureus) were isolated from the oral cavity and/or stomach of four false-positive UBT cases. The in vitro urease activity of these non-H. pylori bacteria was weaker than that of H. pylori. There were no significant differences in the urease activity of urease-positive non-H. pylori bacteria between false-positive and negative UBT cases. In addition, no significant correlations between UBT value and in vitro urease activity were observed among urease-positive non-H. pylori strains. These results suggest that the in vitro urease activities of urease-positive non-H. pylori bacteria are not related to the false-positive UBT results. As the number of urease-positive non-H. pylori bacteria was not determined in the gastric biopsy specimens in the present study, further quantitative studies of such urease-positive bacteria are needed.

The urease activity of non-H. pylori bacteria was markedly inhibited at strongly acidic pH values. Husebye et al. (1992) reported that fasting hypochlorhydria with Gram-positive gastric flora was prevalent in healthy old people. In the present study, false-positive results on UBT were seen in three patients (cases 1, 2 and 4) over 70 years of age. As gastric acid secretion is often suppressed due to the progression of mucosal atrophy in elderly patients (all four patients with false-positive UBT suffered from atrophic gastritis in the present study), it is likely that urease-positive non-H. pylori Gram-positive bacteria, such as staphylococci, were responsible for the false-positive results in the UBT. Urita et al. (2006) reported that colonic bacterial ureolysis influences the results of UBT’s; a duodenal UBT in which 20 ml water containing 100 mg [13C]urea was sprayed into the duodenum gave positive results in 6/143 H. pylori-negative patients. It is also

Table 3. In vitro urease activity of bacterial isolates in UBT negative and false-positive cases

<table>
<thead>
<tr>
<th>Urease activity (U mg⁻¹)</th>
<th>Isolates from negative UBT patients*</th>
<th>Isolates from false-positive UBT patients†</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1</td>
<td>15 (4)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>0.1–1</td>
<td>9 (0)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>0.01–0.99</td>
<td>6 (0)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>0.001–0.0099</td>
<td>9 (2)</td>
<td>1 (0)</td>
</tr>
</tbody>
</table>

*A total of 59 strains were isolated as urease-positive bacteria from negative UBT patients, but urease activity was only quantified for 39 strains due to unsuccessful subculture of the remaining 20 strains. The number of bacterial strains isolated from the stomach is shown in parentheses.

†A total of 12 strains were isolated as urease-positive bacteria from false-positive UBT patients, but urease activity was only quantified for eight strains due to unsuccessful subculture of the remaining four strains. The number of bacterial strains isolated from the stomach is shown in parentheses.
possible that the gastric urease-positive non-*H. pylori* bacteria detected in our study originated from the duodenum or small intestine under hypochlorhydric conditions. Similarly, urease-positive non-*H. pylori* bacteria in the oral cavity might be responsible for the false-positive reactions in the UBT after their translocation from the oral cavity to the gastric mucosa in hypochlorhydria.

**Implications of the experimental data**

Film-coated $^{13}$C urea tablets were used for the UBT in this study. It is likely that non-*H. pylori* urease-positive bacteria in the stomach rather than in the oral cavity caused the false-positive reactions, as UBT values at 20 min were significantly higher than those at 5 min.

In one of five patients showing borderline results in the UBT (4.2%) (Table 1), urease-positive non-*H. pylori* bacteria were not detected and an RUT using Helico-check was positive. This patient was diagnosed as *H. pylori*-positive in the present study. An RUT can be evaluated within 2 h because of the strong urease activity of *H. pylori*. In this study, the in vitro urease activity detected using an ammonia test for *H. pylori* strains was significantly higher than that of other urease-positive non-*H. pylori* strains (Table 4). In addition, Vaira et al. (1988) reported that non-*H. pylori* urease-producing bacteria at a concentration of $10^7$ c.f.u. ml$^{-1}$ did not give a positive result in an RUT with at least a 1 h incubation period at either room temperature or 50 °C. However, there is a possibility that a false-positive reaction in the RUT was induced in the patient.

As administration of gastric acid inhibitors or antibiotics changes the gastric environment, diagnosis of *H. pylori* infection in patients treated with these drugs needs to be considered carefully. It is difficult to assess the influence of urease-producing bacteria other than *H. pylori* in the stomach in the results of a UBT. Hypochlorhydric conditions in the gastric mucosa of elderly patients accelerate colonization and overgrowth of non-*H. pylori* urease-positive bacteria. As it seems that the false-positive results in UBTs are dependent on colonization of urease-positive non-*H. pylori* bacteria and hypochlorhydric conditions.

![Fig. 1.](image) **Fig. 1.** *In vitro* urease activity of urease-positive bacteria other than *H. pylori* isolated from the stomach (a) and oral cavity (b) in false-positive and negative UBT patients. The oral urease-positive non-*H. pylori* bacteria were isolated from swabs obtained from within the oral cavity. The *in vitro* urease activity of *P. mirabilis* isolated from the oral cavity (first and second UBT) and stomach (second UBT) in case 1 and *K. pneumoniae* isolated from the oral cavity (second UBT) in case 2 was not tested due to unsuccessful subculturing. In total, 71 strains were isolated as urease-positive bacteria from negative and false-positive UBT patients, but urease activity was only quantified for 47 strains due to unsuccessful subculture of the remaining 24 strains.

![Fig. 2.](image) **Fig. 2.** Correlation between UBT value and *in vitro* urease activity of urease-positive bacteria other than *H. pylori* isolated from the stomach and oral cavity. In total, 47 strains of urease-positive bacteria isolated from the oral cavity (37 strains: four and 33 from false-positive and negative cases, respectively) and stomach (ten strains: four and six from false-positive and negative cases, respectively) were evaluated for *in vitro* urease activity, as described in Methods.
Table 4. Urease activity of various isolated bacteria under acidic conditions

The urease activity of *P. mirabilis* isolated from the oral cavity (first UBT and second UBT) and stomach (second UBT) in case 1, and *K. pneumoniae* and *E. cloaca* isolated from the oral cavity (second UBT) in case 2, was not tested due to unsuccessful subculturing.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Urease activity (NH₃ production min⁻¹)¹*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 1</td>
</tr>
<tr>
<td><em>S. aureus</em> 1†</td>
<td>166.4 (40.1)</td>
</tr>
<tr>
<td><em>S. aureus</em> 2‡</td>
<td>76.4 (60.4)</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>125.5 (10.1)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>30.9 (43.0)</td>
</tr>
<tr>
<td><em>C. freundii</em></td>
<td>15.5 (60.7)</td>
</tr>
<tr>
<td><em>H. pylori</em> TK1402</td>
<td>1496.4 (92.5)</td>
</tr>
<tr>
<td><em>H. pylori</em> TK1029</td>
<td>1842.7 (93.7)</td>
</tr>
</tbody>
</table>

*The relative urease production (%) is shown in parentheses.
†Isolated from case 3.
‡Isolated from case 4.

conditions in the gastric mucosa, attention should focus on the influence of stomach bacteria.

REFERENCES


