Enhancement of Amoxicillin Resistance after Unsuccessful Helicobacter pylori Eradication

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A high rate of resistance (49.5 to 72.7%) to amoxicillin (AMX) was observed in Helicobacter pylori after two or three unsuccessful eradication attempts. Unsuccessful eradication regimens significantly increase resistance to not only clarithromycin (CLR) and metronidazole (MNZ) but also AMX.

Currently available eradication regimens for Helicobacter pylori are triple-drug combination regimens comprising a proton pump inhibitor (PPI) and two antibiotic drugs, and clarithromycin (CLR), metronidazole (MNZ), and amoxicillin (AMX) are commonly used antibiotics (12). Although H. pylori bacteria easily become resistant to CLR and MNZ, H. pylori has been thought to seldom become resistant to AMX (6). In the present study, the resistance rates after unsuccessful eradication attempts were examined.

A total of 343 patients (189 males and 154 females; mean age, 55.8 years) with H. pylori infection were enrolled between September 2004 and October 2010. H. pylori infection was defined by H. pylori culture positivity. Of the total, 22 patients had no history of antibacterial therapy for eradication, 211 patients had one treatment failure, 99 patients had two treatment failures, and 11 patients had three treatment failures (first-line treatment, triple therapy with CLR [800 mg/day], AMX [1,500 mg/day], and PPI for 7 days; second-line treatment, triple therapy with MNZ [500 mg/day], AMX [1,500 mg/day], and PPI for 7 days; third-line treatment, triple therapy with fluoroquinolone [levofloxacin, 400 mg/day; gatifloxacin, 400 mg/day; or sitafloxacin, 400 mg/day], AMX [2,000 mg/day], and PPI for 7 days) (8, 13). All patients underwent esophagogastroduodenoscopy and gastric biopsy for bacterial culture 6 to 12 months after the eradication failure at Keio University Hospital and National Tokyo Medical Center.

### Table 1. Eradication failures and resistance rates

<table>
<thead>
<tr>
<th>Agent</th>
<th>Prior treatment</th>
<th>With AMX MIC (µg/ml) of:</th>
<th>Other resistance</th>
<th>MIC of agent</th>
<th>50%</th>
<th>90%</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≥0.06</td>
<td>≥0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMX</td>
<td>None</td>
<td>13.6 (3/22)</td>
<td>0 (0/22)</td>
<td>&lt;0.015</td>
<td>0.06</td>
<td>&lt;0.015–0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>One failure</td>
<td>26.5 (56/211)</td>
<td>0.9 (2/211)</td>
<td>&lt;0.015</td>
<td>0.12</td>
<td>&lt;0.015–0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Two failures</td>
<td>49.5 (49/99) ++ ###</td>
<td>6.1 (6/99) #</td>
<td>0.03</td>
<td>0.25</td>
<td>&lt;0.015–4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Three failures</td>
<td>72.7 (8/11) +++ # # #</td>
<td>18.2 (2/11) # #</td>
<td>0.12</td>
<td>0.5</td>
<td>&lt;0.015–4</td>
<td></td>
</tr>
<tr>
<td>CLR</td>
<td>No treatment</td>
<td>9.1 (2/22)</td>
<td>0.03</td>
<td>0.25</td>
<td>&lt;0.015–8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>One failure</td>
<td>89.6 (189/211) ++</td>
<td>16</td>
<td>32</td>
<td>&lt;0.015–64</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Two failures</td>
<td>88.8 (88/99) +++</td>
<td>16</td>
<td>32</td>
<td>&lt;0.015–64</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Three failures</td>
<td>72.7 (8/11) +++</td>
<td>16</td>
<td>64</td>
<td>&lt;0.015–64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MNZ</td>
<td>None</td>
<td>13.6 (3/22)</td>
<td>2</td>
<td>8</td>
<td>0.25–32</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>One failure</td>
<td>4.7 (10/211)</td>
<td>1</td>
<td>2</td>
<td>0.5–32</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Two failures</td>
<td>72.7 (72/99) +++ ###</td>
<td>16</td>
<td>64</td>
<td>1–64</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Three failures</td>
<td>72.7 (8/11) +++ # # #</td>
<td>16</td>
<td>32</td>
<td>4–32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* AMX resistance, MIC ≥ 0.06 µg/ml; AMX high-level resistance, MIC ≥ 0.5 µg/ml; CLR resistance, MIC ≥ 1 µg/ml; MNZ resistance, MIC ≥ 8 µg/ml. ++, P < 0.01 versus results for nontreatment group; ++++, P < 0.001 versus results for nontreatment group; #, P < 0.05 versus results for one-failure group; ###, P < 0.01 versus results for one-failure group.

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Susceptibilities of *H. pylori* isolates to AMX, CLR, and MNZ were determined by the agar dilution method according to the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) (1, 7). Isolates were considered resistant to MNZ if the MIC of the drug was ≥8 µg/ml and to CLR if the MIC was ≥1 µg/ml (9). For AMX, the interpretive standard (susceptible, ≤0.03 µg/ml) established by the Japanese Society of Chemotherapy was used (3). Isolates were defined as high-level resistant and resistant to AMX if the MIC was ≥0.5 µg/ml and ≥0.06 µg/ml, respectively (11), in this study. Differences between groups were compared by Fisher’s exact test or the chi-squared test.

The rates of resistance to AMX in the groups with no history of eradication treatment, one treatment failure, two treatment failures, and three treatment failures were 13.6%, 26.5%, 49.5%, and 72.7%, respectively. The high-level rates of resistance to AMX in the group with no history of eradication treatment, one treatment failure, two treatment failures, and three treatment failures were 0%, 0.9%, 6.1%, and 18.2%, respectively (Table 1). The rates of resistance to AMX in the group with two treatment failures and that with three treatment failures were significantly higher than that in the group with no history of eradication treatment and that with one treatment failure. To the best of our knowledge, the present study is the first to report the increase in rates of resistance to AMX after unsuccessful *H. pylori* eradication.

The MIC₉₀ of AMX showed 2-fold increases with every eradication failure. The MIC₉₀ of CLR showed 0 to 2 substitutions. The accumulation of higher than that in the strains susceptible to MNZ (Table 3). The AMX resistance rate in the strains resistant to CLR or MNZ was significantly higher than that in the strains susceptible to CLR and MNZ. The rate of resistance to AMX in the strains resistant to both CLR and MNZ was significantly higher than that in the strains susceptible to both CLR and MNZ. The rate of resistance to AMX in the strains resistant to both CLR and MNZ was significantly higher than that in the strains susceptible to MNZ (Table 3).

Efflux pump systems in bacteria, which can eject drugs and toxic compounds, including antibiotics, have a critical role in the development of multidrug resistance. We recently reported that the expression of the TolC efflux pump (*hfa*) was significantly increased under MNZ exposure (14). The efflux pump of *H. pylori* is also associated with the development of resistance to CLR, in addition to 23S rRNA point mutations (2). In addition to the known mutations in the gene coding for PBP, activated efflux systems may also play a role in *H. pylori* resistance to AMX.

In conclusion, contrary to our expectations, resistance to AMX in *H. pylori* was gradually induced after unsuccessful eradication attempts. The data are clearly consistent with the association of resistance rates and eradication failures. If AMX-resistant *H. pylori* strains were to spread further, serious problems would arise, resulting in increasing eradication failures (10). Our results suggest that clinicians should be aware of
AMX resistance together with resistance to other antibiotics in the future.

REFERENCES

ERRATUM

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Volume 55, no. 6, p. 3012–3014, 2011. Page 3013, column 2, line 4: The reverse primer sequence should read 5’-CGCTATCGTCTGT TCTTTTGGG-3’.