Antibiotic Resistance of *Helicobacter Pylori* Isolated in the Southeast Coastal Region of China

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Keywords

*Helicobacter pylori*, antibiotic resistance, clarithromycin, metronidazole, levofloxacin, amoxicillin.

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**Abstract**

**Background:** The resistance of *Helicobacter pylori* (*H. pylori*) to antibiotics is increasing worldwide, lowering its efficacy in current eradication therapies. This study evaluated *H. pylori* resistance to antibiotics in the southeast coastal region of China and suggests appropriate alternatives.

**Materials and Methods:** Seventeen thousand seven hundred and thirty one *H. pylori* strains were collected from eight areas of two provinces in coastal southeast China from 2010 to 2012. The resistance of these strains to six antibiotics was tested using the agar dilution method.

**Results:** The resistance rates to clarithromycin, metronidazole, levofloxacin, amoxicillin, gentamicin and furazolidone were 21.5, 95.4, 20.6, 0.1, 0.1 and 0.1%, respectively. Double, triple and quadruple antibacterial resistance percentages were 25.5, 7.5 and 0.1%, respectively. A positive association between the resistance to levofloxacin and to clarithromycin was found, but there was a negative correlation in the resistances to levofloxacin and to metronidazole.

**Conclusions:** The prevalence of *H. pylori* resistance to clarithromycin, metronidazole, levofloxacin and multiple antibiotics in coastal southeast China is high. Choice of therapy should be individualized based on a susceptibility test in this region of the country.

*Helicobacter pylori* (*H. pylori*) is the main pathogen of gastric diseases [1]. The first-line treatment for *H. pylori* infections is a triple eradication therapy consisting of one proton pump inhibitor (PPI) and two antibiotics [2,3]. However, the progressive increase in the resistance to antibiotics of *H. pylori* has caused more and more eradication failures in both children and adults [4–6]. To ensure eradication rate exceeding 80%, prior susceptibility tests have been recommended in high resistance (clarithromycin >15–20%) areas [2]. Although other treatments such as sequential therapy or concomitant therapy have achieved acceptable eradication rates with the use of more antibiotics [7], in recent years, there have been reports from several countries of a high percentage of *H. pylori* clinical isolates with triple and quadruple resistance [8–10], indicating that *H. pylori* treatment has become a great challenge.

The widespread and indiscriminate use of antibiotics in China is the primary reason for *H. pylori* resistance
and eradication failure [11]. To combat this problem, the therapy given to each person should be customized, that is, prior to treatment a susceptibility test should be performed to ensure that the strains of the infected person are sensitive to the prescribed antibiotics. This should be imperative.

The southeastern coastal region of China is relatively developed and suitable for promoting individualized treatment. Most of the previous relevant literature concerning this region is from a single small area and do not include a large number of samples. Therefore, comprehensive epidemiologic surveillances of H. pylori resistances to antibiotics are urgently required. In this study, we conducted a large population-based investigation in multiple areas of coastal southeast China employing antibiotic susceptibility tests for metronidazole, levofloxacin, clarithromycin, amoxicillin, gentamicin, and furazolidone to determine the status of H. pylori antibiotic resistance and resistance patterns.

Materials and Methods

Isolation of H. Pylori Strains

Gastric mucosa biopsy samples taken from the antrum [12] were preserved in the brain-heart infusion broth (Oxoid, Dardilly, France) with 5% glycerin and sent to the laboratory of Hangzhou Zhiyuan Medical Inspection Institute. The homogenate of stomach biopsy specimens was inoculated onto Columbia agar plates (Oxoid) supplemented with 5% fresh defibrinated sheep blood and kept under microaerophilic conditions (5% O2, 10% CO2 and 85% N2) at 37 °C for 3 days. Colonies displaying typical H. pylori morphology were selected and identified by Gram staining and urease, oxidase, and catalase activity testing.

From January 2010 to April 2012, a total of 17,731 H. pylori strains were isolated from 51,891 patients. The samples were collected from 22 hospitals in eight areas of two southeast coastal provinces of China: 1, in Zhejiang province, the cities Hangzhou, Jiaxing, Jinhua, Taizhou, Wenzhou, and Zhoushan and Shangyu county; and 2, in Jiangsu province, Wuxi city (Fig. 1). All patients initially presented with upper gastrointestinal symptoms and had undergone upper gastrointestinal endoscopy. This study was approved by the Ethics Committee of Chinese Center for Disease Control and Prevention, and every patient provided written informed consent.

Antibiotic Susceptibility Testing

Susceptibility of H. pylori to six antibiotics (metronidazole, levofloxacin, clarithromycin, amoxicillin, gentamicin, and furazolidone) was tested via agar dilution method using reference standards obtained from the National Institutes for Food and Drug Control. Ten-microliter suspensions (10^8 CFU/mL) of each isolate from a mixture of colonies in brain-heart infusion broth (Oxoid) were inoculated onto Mueller-Hinton agar plates (Oxoid) that included 5% sheep blood and a single antibiotic and incubated at 37 °C for 3 days under microaerophilic conditions. The resistance break points to metronidazole, levofloxacin, clarithromycin, amoxicillin, gentamicin, and furazolidone were set at ≥8, ≥2, ≥1, ≥2, ≥16, and ≥2 µg/mL [13–16], respectively. ATCC 43504 (NCTC11637) was used as the control strain. All the tests were conducted at Hangzhou Zhiyuan Medical Inspection Institute.

Statistical Analysis

Statistical analyses were carried out using SPSS statistical software package version 18.0 (SPSS Inc., Chicago, IL, USA). Differences in resistance rates among different years and antibiotic resistance groups were assessed with the chi-squared (χ^2) test. A probability (P) value ≤ .05 was considered statistically significant.

Results

The resistance rates of H. pylori to clarithromycin, metronidazole, levofloxacin, amoxicillin, gentamicin, and furazolidone were 21.5, 95.4, 20.6, 0.1, 0.1, and 0.1%, respectively. No noticeable upward or downward trend in resistance rates to these antibiotics was found from 2010 to 2012.
Of the 17,731 H. pylori strains, 5872 (33.1%) were resistant to more than one antibiotic (25.5% for double resistance, 7.5% for triple, and 0.1% for quadruple resistance). Eighteen patterns (6 double, 8 triple, and 4 quadruple) of multiple resistance were found (Table 1). The predominant three multiple resistance patterns were metronidazole + clarithromycin, metronidazole + levofloxacin, and metronidazole + levofloxacin + clarithromycin. The most prominent patterns were metronidazole + clarithromycin and metronidazole + levofloxacin in double resistance, metronidazole + levofloxacin + clarithromycin in triple resistance, and metronidazole + levofloxacin + clarithromycin + furazolidone (70.0%, 7/10) in quadruple resistance (Fig. 2).

We found that the resistance rate to levofloxacin was significantly higher in those H. pylori strains that were also resistant to clarithromycin (36.5%) compared with strains that were sensitive to clarithromycin (16.3%, p < .001). Yet, the prevalence of levofloxacin resistance was significantly lower in those strains resistant to metronidazole (20.4%) compared with the strains sensitive to metronidazole (24.9%, p < .001).

Table 1 Result of susceptibility test of 17,731 Helicobacter pylori strains

<table>
<thead>
<tr>
<th>Susceptibility test results</th>
<th>No. of strains</th>
<th>Resistance rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive to all ABs</td>
<td>521</td>
<td>2.9</td>
</tr>
<tr>
<td>MTZ</td>
<td>16,908</td>
<td>95.4</td>
</tr>
<tr>
<td>LVX</td>
<td>3661</td>
<td>20.6</td>
</tr>
<tr>
<td>CLR</td>
<td>3810</td>
<td>21.5</td>
</tr>
<tr>
<td>GEN</td>
<td>17</td>
<td>0.1</td>
</tr>
<tr>
<td>AMX</td>
<td>22</td>
<td>0.1</td>
</tr>
<tr>
<td>FR</td>
<td>21</td>
<td>0.1</td>
</tr>
<tr>
<td>MTZ + LVX</td>
<td>2127</td>
<td>12.0</td>
</tr>
<tr>
<td>MTZ + CLR</td>
<td>2307</td>
<td>13.0</td>
</tr>
<tr>
<td>LVX + CLR</td>
<td>73</td>
<td>0.4</td>
</tr>
<tr>
<td>MTZ + AMX</td>
<td>4</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>MTZ + GEN</td>
<td>5</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>MTZ + FR</td>
<td>9</td>
<td>0.1</td>
</tr>
<tr>
<td>MTZ + LVX + CLR</td>
<td>1307</td>
<td>7.4</td>
</tr>
<tr>
<td>MTZ + LVX + AMX</td>
<td>4</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>MTZ + CLR + AMX</td>
<td>12</td>
<td>0.1</td>
</tr>
<tr>
<td>MTZ + LVX + GEN</td>
<td>7</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>MTZ + CLR + GEN</td>
<td>2</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>LVX + CLR + GEN</td>
<td>1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>MTZ + LVX + FR</td>
<td>2</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>MTZ + CLR + FR</td>
<td>2</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>MTZ + CLR + AMX + FR</td>
<td>1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>MTZ + LVX + CLR + FR</td>
<td>7</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>MTZ + LVX + CLR + GEN</td>
<td>1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>MTZ + LVX + CLR + AMX</td>
<td>1</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

MTZ, metronidazole; LVX, levofloxacin; CLR, clarithromycin; AMX, amoxicillin; GEN, gentamicin; FR, furazolidone; AB, antibiotic.

Resistance rates of H. pylori strains collected across the eight geographic areas were 93.5–96.5% to metronidazole, 16.3–29.4% to levofloxacin, 17.1–26.3% to clarithromycin, and 26.2–36.1% to more than one antibiotic (Table 2).

Discussions

The antibiotic resistance of H. pylori is increasing around the world and has caused a loss in the effectiveness of the current therapeutic regimens [17]. Clarithromycin is a basic antibiotic in eradication therapy, and treatment failure is highly related to its resistance of H. pylori [18–21]. In the present large-sample study, high resistance rates to the antibiotics clarithromycin, metronidazole, and levofloxacin were observed for the years 2010 to 2012 in southeast coastal provinces of China.

The overall resistance rate to clarithromycin was 21.5%, which is slightly higher than previous reports [22,23]. Members of the macrolide antibiotics are usually used to treat respiratory infections, especially for children, and their widespread clinical use has led to an increase in resistance rates [6,24]. Because the resistance rate to clarithromycin in the present study was
higher than 20%, a susceptibility test is recommended prior to clarithromycin-based antibiotic treatment. Alternatively, a quadruple regimen containing bismuth, sequential, or “concomitant” therapy without clarithromycin is an option for first-line treatment, according to the Maastricht IV (Consensus Report of the European Helicobacter Study Group) [2].

The global resistance rate of H. pylori to metronidazole was found to range from 14.4 to 93.2% [17]. Due to the overuse of metronidazole in developing countries, the resistance rates to this antibiotic differ significantly between the developed and developing countries [25]; the resistance rate in China was reported to be from 11.4 to 99.1% [26–29]. In the present study, we found a resistance rate close to 100% (95.4%). But because of the medicinal property of the metronidazole [2], we think the increasing dosage and treatment duration will help to increase the eradication rate in this region.

Several published guidelines have recommended levofloxacin as second-line therapy [2,30–32]. However, its resistance rate in the present research reached 20.6%. The prevalence of levofloxacin resistance increased worldwide as it has been used widely to treat urinary infections during the last two decades [17]. In Asia, levofloxacin resistance rates in Japan (14.9%) and South Korea (12.4%) exceeded that of Malaysia (0%) [33–35]. A high resistance rate (50.3%) was also reported in China [26]. Although levofloxacin-based triple and sequential therapy were reported to result in better cure rates than clarithromycin-based therapies [36,37], rising rates of levofloxacin resistance should be taken into account. Considering that the resistance rate to levofloxacin in this study was greater than 20%, levofloxacin-based therapies should not be recommended as first-line treatment. So, taking levofloxacin as a second-line drug may be reasonable or a prior susceptibility test is also an option.

Our study revealed that 33.1% of the isolated strains were resistant to more than one antibiotic. The main resistance patterns were metronidazole + clarithromycin (13.0%), metronidazole + levofloxacin (12.0%), and metronidazole + levofloxacin + clarithromycin (7.4%), which included the high resistance antibiotics clarithromycin, metronidazole, and levofloxacin. Several quadruple resistant patterns were found, consisting of different combinations of the six-first- or second-line treatment antibiotics (clarithromycin, metronidazole, levofloxacin, amoxicillin, gentamicin, and furazolidone). This suggests that without the guidance provided by susceptibility test data due to multidrug resistance, about one-third of patients will experience eradication therapy failure, even if they repeat treatment with different therapeutic regimes. Therefore, empirical therapy and blindly changing prescriptions by increasing the variety of drugs or using alternative antibiotics are not reasonable for these people. For this reason, we recommend individualized therapy based on the result of a susceptibility test.

In agreement with prior reports regarding the promotion of resistance to different antibiotics mutually in the same strain [38,39], a positive correlation was found between levofloxacin and clarithromycin resistance in our study. Nevertheless, a negative relationship between levofloxacin and metronidazole was also observed. As in other studies, this might partially be due to antibiotic consumption in a particular geographic area [40–43]. Consumption of the three main antibiotics (clarithromycin, metronidazole, and levofloxacin) that were shown to have high resistance rates in the H. pylori strains of our study may have been influenced by their cost and differences in consumer incomes. High-income groups may prefer the more expensive medicines, such as clarithromycin and levofloxacin, to that of metronidazole.

From 2010 to 2015, in Zhejiang province, the incomes
of people in urban areas was predicted to be 2.5-fold than that of rural people [44]. Therefore, the city hospital may have more high-income patients, while there are more low-income patients in a county hospital. Physicians should recommend that patients in this region take seldom-used antibiotics to prevent resistance or employ the three low-resistance antibiotics, amoxicillin (0.1%), gentamicin (0.1%), and furazolidone (0.1%), as the first-line choice for H. pylori infection treatment.

Conclusion

In this study, a large number of H. pylori strains isolated from coastal southeast China were shown to be resistant to clarithromycin, metronidazole, levofloxacin, or more than one antibiotic. Because resistance to antibiotics has become severe in this region of the country, it is reasonable and imperative to individualize therapy based on susceptibility testing or choose the low resistance antibiotics, amoxicillin, gentamicin, and furazolidone, in H. pylori eradication therapy.

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Competing interests: The authors have no competing interests.

Author Contributions

Peng Su took part in the isolation and susceptibility test of all the strains and had principal responsibility for data analysis. Jianzhong Zhang and Youming Li designed and supervised the study. Strain isolation, susceptibility test, and data integration were taken care by Ningmin Yang and Peng su. Sample collection was done by Youming Li, Hongzhang Li, Jiakun Zhang, Lang Lin, Qunying Wang, Feng Guo, Zizhong Ji, Jibo Mao, Wuheng Tang, Zhengchao Shi, Wei Shao, Junliang Mao, Xinjian Zhu, Xiaofeng Zhang, Yuelfeng Tong, Huimin Tu, Mizu Jiang, Zhiyong Wang, and Fengze Jin.

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