

## RESEARCH ARTICLE

# Antibiotic resistance of *Helicobacter pylori* to 16 antibiotics in clinical patients

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**Background:** Resistance of *Helicobacter pylori* (*H. pylori*) to antibiotics is increasing worldwide. To determine the status of *H. pylori* resistance and its patterns in clinical patients, an investigation utilizing susceptibility testing for commonly used antibiotics was needed.

**Methods:** Total of 2283 *H. pylori* strains were collected from 2013 to 2016. The resistance and its patterns of these strains were tested by agar dilution method. The resistance rate and minimal inhibition concentration (MIC) in different gender groups were also analyzed.

**Results:** The overall resistance rates were as following: amoxicillin (1.58%), clarithromycin (22.73%), levofloxacin (24.75%), furazolidone (1.49%), doxycycline (9.20%), cefetamet (97.20%), ceftriaxone (49.60%), cefuroxime (25.20%), gentamicin (3.73%), azithromycin (85.60%), rifampicin (2.80%), metronidazole (92.53%), ornidazole (94.27%), tinidazole (87.20%), ciprofloxacin (43.20%), and moxifloxacin (38.53%). There were only 64.08% strains pan-susceptible to amoxicillin, clarithromycin, levofloxacin, and furazolidone, followed by mono resistance (23.17%), double resistance (11.13%), triple resistance (1.36%), and quadruple resistance (0.26%). Significant differences in the resistance rate and MIC were also observed in different gender groups.

**Conclusion:** Antibiotic resistance trends of *H. pylori* is increasing in clinical patients. With the increasing resistance, it is imperative to individualized therapy based on the results of drug susceptibility testing.

**KEYWORDS**

antibiotic resistance, gender, *Helicobacter pylori*

## 1 | INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is a gram-negative and microaerophilic bacterium, which as a kind of common pathogenic bacteria widely spread among the population, especially in developing countries.<sup>1,2</sup> Over the past 30 years, *H. pylori* has been recognized as one of the main pathogenic factors in the occurrence and development of gastrointestinal diseases such as gastric cancer, chronic gastritis, peptic ulcer, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and it been categorized as the class I carcinogen by World Health Organization.<sup>3,4</sup> Moreover, *H. pylori* infection has also been

observed to be associated with diseases of nervous system, circulatory system, and blood system.<sup>5-7</sup> Eradication of *H. pylori* infection is directly related to the prognosis of the aforementioned diseases. Clinical research studies have demonstrated that *H. pylori* eradication will uncontroverted benefit those infected patients because it can not only promote gastric mucosa healing but also reduce the main risk factor of MALT lymphoma and gastric cancer.<sup>8,9</sup> Therefore, *H. pylori* eradication is of great significance in clinical practice.

Regimens that the combination of proton pump inhibitors (PPIs) and 2 antibiotics as the first-line treatment for *H. pylori* eradication have been used worldwide in recent years.<sup>10</sup> Unfortunately, the

success rate of eradication has been seriously limited for the progressive increase in antibiotic resistance.<sup>11</sup> Thus, in order to determine the status of *H. pylori* resistance and its patterns in patients and reliably select sensitive antibiotics for clinical use, it is necessary to perform an investigation especially in the absence of individualized *H. pylori* culture and antibiotic susceptibility test.

In this study, a large sample-based investigation that utilizing drug susceptibility testing for 16 commonly used antibiotics in 5 areas of Zhejiang Province, China, was performed to determine the status of *H. pylori* resistance and its patterns. Moreover, the resistance rate and the minimal inhibition concentration (MIC) value of antibiotics in different gender groups were also analyzed.

## 2 | MATERIALS AND METHODS

### 2.1 | Patient collection and exclusion criteria

Patients in this study were collected from 5 hospitals in 5 areas of Zhejiang Province, China, from 2013 to 2016. They are Taibao Hospital, Kangyang Hospital, the second Hospital of Beilun, the sixth Hospital of Cixi, and the Affiliated Hospital of Ningbo University School of Medicine. These patients included in the study were all older than 4 18 years and diagnosed with *H. pylori* infection by<sup>12</sup> C-labeled urea breath test. Evaluated by clinical gastroenterologists, the patients who had endoscopy contraindications or received bismuth salts, PPIs, antibiotics, or H<sub>2</sub> receptor antagonists within the last 4 weeks were all excluded from this study.<sup>13</sup> Two gastric mucosal biopsy specimens (from the antrum and corpus) were collected from patients undergoing upper endoscopy and immediately stored in a brain-heart infusion broth (Oxoid, Basingstoke, United Kingdom) with 5% glycerin. Then, they were all transported to the microbiology laboratory in the Affiliated Hospital of Ningbo University School of Medicine for *H. pylori* culture and antibiotic susceptibility testing. This study was approved by the Human Research Ethics Committee of Ningbo University School of Medicine. Written informed consents were obtained from all patients.

### 2.2 | Isolation and identification of *H. pylori* strains

After gastric mucosa biopsy specimen was ground, the homogenate of specimen was inoculated onto modified culture medium (Zhao Tai biotechnology Co., Ltd., Hunan, China). Then, the plate was cultured

under a microaerophilic condition (85% nitrogen, 10% carbon dioxide, and 5% oxygen) at 37°C for 2-4 days. Total of 2283 *H. pylori* strains in this study were identified by Gram staining, positive reactions of urease, catalase, and oxidase test.

### 2.3 | Antibiotic susceptibility test

Antibiotic susceptibility of the isolated *H. pylori* strains to 16 antibiotics (amoxicillin, clarithromycin, levofloxacin, furazolidone, azithromycin, cefetamet, ceftriaxone, cefuroxime, doxycycline, gentamicin, rifampicin, metronidazole, ornidazole, tinidazole, ciprofloxacin, and moxifloxacin) was tested by the agar dilution method. Three microliters *H. pylori* suspensions (about 10<sup>8</sup> CFU/mL, determined by McFarland standards) was transferred onto Mueller-Hintonagar (Oxoid) containing 5% sheep blood and a single antibiotic. Then, they were all cultured under a microaerophilic condition at 37°C for 3 days. The resistance breakpoints of each antibiotics are referred to previous reports or the Clinical and Laboratory Standards Institute (CLSI) guidelines,<sup>13</sup> and showed in Table S1. NCTC11637 *H. pylori* strains was used as a positive control. All tests were repeated once.

### 2.4 | Statistical analysis

All statistical analyses in this study were performed with Statistical Product and Service Solutions (SPSS) 19.0 software (SPSS, Chicago, IL, USA). Differences of resistance rates in different gender groups were assessed with the  $\chi^2$  test, whereas the MIC value of different groups were assessed with Student's *t* test. *P* < .05 was regarded as statistically significant.

## 3 | RESULTS

### 3.1 | Determination of resistance in 4 most commonly used antibiotics

Amoxicillin, clarithromycin, levofloxacin, and furazolidone are the most frequently used antibiotics to constitute the first-line treatment for *H. pylori* eradication in China nowadays.<sup>12,14</sup> To understand the latest regional resistance of *H. pylori* to these 4 most frequently used antibiotics, we performed an investigation based on 2283 *H. pylori* strains isolated from clinical patients. As shown in

**TABLE 1** Resistance rates of *Helicobacter pylori* strains between male and female

Antibiotics	No. of resistance strains (%)			<i>P</i>	MIC ( $\bar{x} \pm s$ )			<i>P</i>
	Male (n = 1225)	Female (n = 1058)	Total (n = 2283)		Male (n = 1225)	Female (n = 1058)	Total (n = 2283)	
AMX	21 (1.71)	15 (1.42)	36 (1.58)	.616	10.472 ± 6.642	10.679 ± 6.166	10.571 ± 6.411	.767
CLR	246 (20.08)	273 (25.80)	519 (22.73)	.001	15.695 ± 6.646	16.179 ± 7.247	15.952 ± 6.972	.315
LVX	267 (21.80)	298 (28.17)	565 (24.75)	<.001	12.264 ± 10.169	14.456 ± 11.112	13.430 ± 10.732	<.001
FR	18 (1.47)	16 (1.51)	34 (1.49)	.534	17.410 ± 11.612	17.075 ± 11.667	17.253 ± 11.635	.609

*P*: male group compared with female group.

Table 1, the resistance rates of amoxicillin, clarithromycin, levofloxacin, and furazolidone were 1.58%, 22.73%, 24.75%, and 1.49%, respectively. The resistance rates of clarithromycin and levofloxacin in female group were much higher than those in male group ( $P = .001$  and  $P < .001$ , respectively). Similar to resistance rate, the average MIC value of levofloxacin in female group was also larger than that in male group ( $P < .002$ ). There was no significant difference for amoxicillin and furazolidone in both resistance rate and MIC value.

### 3.2 | Multiple antibiotic resistance

Multidrug resistance is defined as that one bacterial strain is resistant to 2 or more antibiotics.<sup>15</sup> In our study, 2283 *H. pylori* strains were investigated, only 64.08% were susceptible to all 4 tested antibiotics (amoxicillin, clarithromycin, levofloxacin, and furazolidone). Mono resistance (23.17%, 529/2283), double resistance (11.13%, 254/2283), triple resistance (1.36%, 31/2283), and quadruple resistance (0.26%, 6/2283) were also observed. More specifically, the double resistance of *H. pylori* strains to clarithromycin and levofloxacin was the highest (10.16%), followed by the combination of levofloxacin/amoxicillin and clarithromycin/furazolidone with the resistance rates of 0.31% and 0.26%, respectively. The multiple resistance rates in the past 4 years are all shown in Table 2.

### 3.3 | Resistance of *H. pylori* strains to 12 second-line antibiotics

To obtain more information about the resistance of *H. pylori*, other 12 second-line antibiotics were tested in 750 strains which are resistant to one of the first-line drugs at least. As shown in Table 3, the resistance rates of cefetamet, ornidazole, and metronidazole were more than 90%, whereas rifampicin, gentamicin, and doxycycline were still in a low level relatively. The resistance rates of cefetamet ( $P = .046$ ), azithromycin ( $P = .009$ ), metronidazole ( $P = .012$ ), and ciprofloxacin ( $P < .001$ ) in female group were much higher than those in male group (Table 3). Only tinidazole resistance rate in female group was lower than that in male group ( $P = .016$ , Table 3). Moreover, the data showed that there were significant differences in the average MIC value of azithromycin ( $P = .001$ ), metronidazole ( $P = .029$ ), ornidazole ( $P = .049$ ), and tinidazole ( $P = .017$ ) between male and female group.

## 4 | DISCUSSION

The eradication therapy of *H. pylori* is confronted with a series of severe challenges at present. Increasing antibiotic resistance to *H. pylori* infection has become one of the biggest obstacles facing current therapeutic regimens.<sup>16,17</sup> Furthermore, multiple antibiotic-resistance patterns of *H. pylori* strains were also observed. To determine the status of *H. pylori* resistance in clinical patients and reliably select sensitive antibiotics for clinical use, in this study, investigation that employing drug susceptibility testing for 16 antibiotics was performed from 2013 to 2016.

Amoxicillin, clarithromycin, levofloxacin, and furazolidone are the most frequently used antibiotics in the first-line treatment of *H. pylori* nowadays in China.<sup>12,14</sup> Previous studies reported that the resistance rates of *H. pylori* strains collected by different geographic areas in China were 0.01%-6.8% to amoxicillin, 17.6%-37.5% to clarithromycin, 20.6%-33.5% to levofloxacin, and 0.01%-0.1% to furazolidone.<sup>13,18-20</sup> This means China is a highly resistant region of clarithromycin and levofloxacin in *H. pylori* eradication. Our current data showed that the overall resistance rates of amoxicillin, clarithromycin, levofloxacin, and furazolidone were 1.58%, 22.73%, 24.75%, and 1.49%, respectively (Table 1). Obviously, compared with the average levels of past, the resistance rate of furazolidone in this region has changed greatly.

Then, other 12 second-line antibiotics were tested in 750 *H. pylori* strains which are resistant to one of the first-line drugs at least. Our data illustrated that the resistance rates of cefetamet, ornidazole, and metronidazole were more than 90%, whereas rifampicin, gentamicin, and doxycycline were still in a low level relatively (Table 3). According to these data, it is reasonable to speculate that rifampicin and gentamicin could be considered as the optional drugs for the treatment of *H. pylori* eradication in patients with repeated eradication failure.

Antibiotic resistance is closely dependent on its use; and other factors such as age and genders can also affect resistance in clinic.<sup>21,22</sup> Our current study demonstrated that the resistance rates of clarithromycin, levofloxacin, cefetamet, azithromycin, metronidazole, and ciprofloxacin in female group were much higher than those in male group (Tables 1 and 3), whereas tinidazole resistance rate in female group was lower than that in male group (Table 3). Similar to resistance rate, our data also showed that there were significant differences in the average MIC value of levofloxacin, azithromycin, metronidazole, ornidazole, and tinidazole between male and female group (Tables 1 and 3). This phenomenon may be related to hormone, genetic differences,

**TABLE 2** Multiple resistance rate in the past 4 years

Patterns	2013 (n = 458)	2014 (n = 506)	2015 (n = 623)	2016 (n = 696)	Total (n = 2283)
No resistance	319 (69.65%)	341 (67.39%)	388 (62.28%)	415 (59.63%)	1463 (64.08%)
Mono resistance	92 (20.09%)	115 (22.73%)	151 (24.24%)	171 (24.57%)	529 (23.17%)
Double resistance	43 (9.39%)	46 (9.09%)	74 (11.88%)	91 (13.07%)	254 (11.13%)
Triple resistance	2 (0.44%)	3 (0.59%)	9 (1.44%)	17 (2.44%)	31 (1.36%)
Quadruple resistance	2 (0.44%)	1 (0.20%)	1 (0.16%)	2 (0.29%)	6 (0.26%)

**TABLE 3** Resistance rates of *Helicobacter pylori* strains to 12 second-line antibiotics

Antibiotics	No. of resistance strain/total (%)			P	MIC( $\bar{x} \pm s$ )			P
	Male	Female	Total		Male	Female	Total	
DOX	30/369 (8.13)	39/381 (10.23)	69/750 (9.20)	.377	2.600 ± 0.966	2.385 ± 0.768	2.478 ± 0.846	.557
CAT	354/369 (95.93)	375/381 (98.43)	729/750 (97.20)	.046	17.203 ± 21.006	16.720 ± 20.260	16.955 ± 20.584	.855
CRO	195/369 (52.85)	177/381 (46.46)	372/750 (49.60)	.093	9.631 ± 15.354	12.542 ± 19.708	11.016 ± 17.548	.358
CXM	81/369 (21.95)	108/381 (28.34)	189/750 (25.20)	.053	11.556 ± 19.871	17.000 ± 22.549	14.667 ± 21.448	.323
GEN	13/369 (3.52)	15/381 (3.94)	28/750 (3.73)	.848	10.455 ± 5.753	10.680 ± 7.587	10.569 ± 6.735	.794
AZM	303/369 (82.11)	339/381 (88.98)	642/750 (85.60)	.009	41.307 ± 26.720	52.354 ± 21.723	47.140 ± 24.776	.001
RFP	12/369 (3.31)	9/381 (2.36)	21/750 (2.80)	.512	9.500 ± 7.550	6.667 ± 8.083	8.286 ± 7.251	.653
MTZ	332/369 (89.97)	362/381 (95.01)	694/750 (92.53)	.012	53.065 ± 51.534	41.471 ± 46.298	47.184 ± 49.219	.029
ONZ	347/369 (94.04)	360/381 (94.49)	707/750 (94.27)	.876	49.130 ± 49.475	39.145 ± 44.731	44.194 ± 47.387	.049
TNZ	333/369 (90.24)	321/381 (84.25)	324/750 (87.20)	.016	43.750 ± 23.301	49.313 ± 21.349	46.093 ± 22.639	.017
CIP	135/369 (36.59)	189/381 (49.61)	160/750 (43.20)	<.001	15.217 ± 10.804	15.670 ± 11.663	15.475 ± 11.268	.802
MXF	131/369 (35.50)	158/381 (41.47)	289/750 (38.53)	.099	9.522 ± 10.199	12.237 ± 11.538	10.965 ± 10.977	.141

P: male group compared with female group.

and frequency of antibiotic use. Compared with the male group, the female group is more likely to get infectious diseases and more willing to receive antibiotic treatment.

Multiple antibiotic-resistance patterns in *H. pylori* were observed more frequent in various regions of the world.<sup>23,24</sup> China also has many similar reports in recent years.<sup>13,15,20</sup> In this study, we found that only 64.08% strains were susceptible to all 4 tested antibiotics in our study (Table 2). The rates of double and multiple antibiotic resistance were 11.13% and 12.75% (Table 2). The main resistance patterns were clarithromycin+levofloxacin, which indicated that about 35.92% patients' eradication treatment will fail if without drug susceptibility testing. However, it also suggested that the regimen containing the combination of clarithromycin and levofloxacin is not suitable for primary eradication if without individualized antibiotic susceptibility testing. Moreover, with increasing multiple resistance in *H. pylori*, it is also a good choice that individualized therapy based on the results of a susceptibility testing.

Conclusively, antibiotic resistance trend of *H. pylori* in Chinese patients is increasing. With the increasing resistance rate and multiple resistance of *H. pylori* to commonly used antibiotics, it is imperative to individualize therapy based on the results of antibiotic susceptibility test.

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## ETHICS APPROVAL

This study was conducted with the approval of the Human Research Ethics Committee of Ningbo University School of Medicine.

## CONFLICT OF INTEREST

The authors declare no conflicts.

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#### SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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