

# The Trend of the Multidrug Resistance of *Helicobacter pylori* Strains Isolated in Chengdu

Xiaofang Li<sup>1</sup>, Xiaoxiao Yang<sup>2</sup>, Lin Zhang<sup>1</sup>, Yu Zhang<sup>1</sup>, Hongmei Wang<sup>1</sup>, Ziyang Zhang<sup>1</sup>, Fangqiong Dong<sup>1</sup>

<sup>1</sup>Gastroenterology Unit, The People's Hospital of Pingchang, Bazhong, China; <sup>2</sup>Yongquan Street Community Health Service Center, Wenjiang District, Chengdu, China

**Correspondence to:** Xiaofang Li, lixiaofang0902@126.com

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## ABSTRACT

**Background:** The treatment of Hp infection has been the most popular topic of the most attention and research by gastrointestinal disease workers, and the biggest problem in the treatment of Hp infection is Hp drug resistance to antibiotics, and Hp drug resistance is the main cause of Hp eradication failure. It is severe for the multidrug resistance of Hp. **Objective:** To investigate the changing trend of *Helicobacter pylori* (Hp) resistance to metronidazole, clarithromycin and amoxicillin in children and adults in Chengdu. **Methods:** The Hp strain determined the minimum antibacterial concentration (MIC) from 585 patients with upper gastrointestinal symptoms, metronidazole, clarithromycin and amoxicillin was measured by the E-test. Clinically isolated Hp strains were obtained by microaerobic condition culture as the diagnostic standard. **Results:** The Hp resistance to metronidazole and clarithromycin in children in Chengdu were: 17.65% (27/153) and 18.06% (78/432) in adults. The Hp resistance to metronidazole and amoxicillin in children in Chengdu were: 11.76% (18/153) and 13.19% (57/432) in adults. The Hp resistance to metronidazole, clarithromycin and amoxicillin in children in Chengdu were: 3.92% (6/153) and 10.41% (45/432) in adults. There were 76.92% of the strains which were resistant to metronidazole, 24.11% to clarithromycin, and 18.46% to amoxicillin. The drug resistance rate of Hp has increased year by year from 2017 to 2021 year, especially the drug resistance rate of Hp to clarithromycin and metronidazole has generally increased. **Conclusions:** The drug resistance rate of Hp has increased year by year from 2017 to 2021 year.

## 1. BACKGROUND

*Helicobacter pylori* (Hp) is an important cause of chronic gastritis which is closely related to the in-

cidence of peptic ulcer. Not only the eradication of Hp can accelerate the healing of peptic ulcer, but also significantly reduce the recurrence rate of ulcer, so that the ulcer disease is cured. In 1994, the World Health Organization's International Cancer Agency (IARC) officially listed Hp as the first carcinogenic factor [1]. Eradication of Hp reduces the recurrence rate after early gastric cancer surgery [1, 2]. Hp infection is closely associated with low-grade malignant gastric mucosa-associated lymphoid tissue (MALT) lymphoma [3-5]. Eradication of Hp reduces lymphoma and even resolves early low-grade malignant B-cell MALT lymphoma. The treatment of Hp infection has been the most popular topic of the most attention and research by gastrointestinal disease workers, and the biggest problem in the treatment of Hp infection is Hp drug resistance to antibiotics [6], and Hp drug resistance is the main cause of Hp eradication failure [7]. It is severe for the multidrug resistance of Hp.

## 2. MATERIAL AND METHODS

### 2.1. Material

The 585 clinically isolated Hp strains were from patients undergoing gastroscopy for upper gastrointestinal symptoms from 2017 to 2021 year, including 153 children (mean age:  $10 \pm 5$  years) without ulcer dyspepsia (69 girls and 84 boys) and 432 adults (mean age,  $52 \pm 17$  years), 144 with nonulcer dyspepsia and 288 with peptic ulcers (220 males and 212 females). All patients were seen in Chengdu and had no history of eradication treatment.

### 2.2. Methods

1) Bacterial culture: using homogenate inoculation,  $37^{\circ}\text{C}$  microaerobic environment, gas extraction-ventilation method, culture for 3 - 7 d, the isolation effect after 3 d of inoculation was observed, and the positive patients were subcultured with bacteria.

2) Identification of Hp strains: strain identification by observing the colony morphology, gram staining, and urease, hydrogen peroxide, and oxidase tests.

3) E-Test: Fresh Hp was collected from 48 to 72 h and diluted to  $3 \times 10^8$  cfu/ml (1 turbidity), absorb 100  $\mu\text{l}$  of diluted bacterial droplets to 90 mm plate, evenly spread with L rod, dry and place antibiotic containing E-test strip (Biochem) in  $37^{\circ}\text{C}$  microaerobic environment, the minimum antibacterial concentration (MIC) was read according to the manufacturer's instructions after 72 h [7, 8].

4) The determine of results: Metronidazole MIC  $\geq 8$  mg/L, clarithromycin MIC  $\geq 1$  mg/L, and amoxicillin MIC  $\geq 0.5$  mg/L were determined as drug-resistant [9, 10].

5) Statistical methods: To calculate and compare the resistance rate of Hp resistance to different antibiotics in different periods, use univariate analysis,  $\chi^2$  test and precise probability method to analyze the relationship between Hp resistance to antibiotics and patient age.

## 3. RESULTS

### 3.1. The Resistance to Metronidazole in Hp

The Hp resistance to metronidazole in children in Chengdu were: 78.43% (120/153) and 76.39% (330/432) in adults. There were 76.92% of the all strains which were resistant to metronidazole. Metronidazole resistance by Hp has significant difference between Children and Adults ( $P = 0.006$ ) (Table 1).

### 3.2. The Resistance to Clarithromycin in Hp

The Hp resistance to clarithromycin in children in Chengdu were: 21.56% (33/153) and 25.00% (1.8/432) in adults. There were 24.11% of the all strains which were resistant to clarithromycin. Clarithromycin resistance by Hp has significant difference between Children and Adults ( $P = 0.015$ ) (Table 1).

### 3.3. The Resistance to Amoxicillin in Hp

The Hp resistance to amoxicillin in children in Chengdu were: 15.68% (24/153) and 19.44% (84/432)

**Table 1.** Susceptibility patterns of *H. pylori* strains isolated from the children and adults.

Antibiotic (s)	No. (%) of resistant isolates				
	Children (n = 153)	Adults (n = 432)	CI (95%)	P	Total (n = 585)
Metronidazole	120 (78.43)	330 (76.39)	0.086 (0.084 - 0.089)	0.006	450 (76.92)
Clarithromycin	33 (21.56)	108 (25)	0.034 (0.030 - 0.037)	0.015	141 (24.11)
Amoxicillin	24 (15.68)	84 (19.44)	0.101 (0.099 - 0.103)	0.017	108 (18.46)
Metronidazole + clarithromycin	27 (17.65)	78 (18.06)	0.067 (0.063 - 0.071)	0.021	105 (17.94)
Metronidazole + amoxicillin	18 (11.76)	57 (13.19)	0.091 (0.090 - 0.092)	0.004	75 (12.82)
Metronidazole + clarithromycin + amoxicillin	6 (3.92)	45 (10.41)	0.023 (0.021 - 0.024)	0.006	51 (8.71)

Differences were very significant for any comparison between children and adults.

in adults. Amoxicillin resistance by Hp has significant difference between children and adults (P = 0.017). There were 18.46% of the all strains which were resistant to amoxicillin (Table 1).

### 3.4. Multiple Drug Resistance in Hp

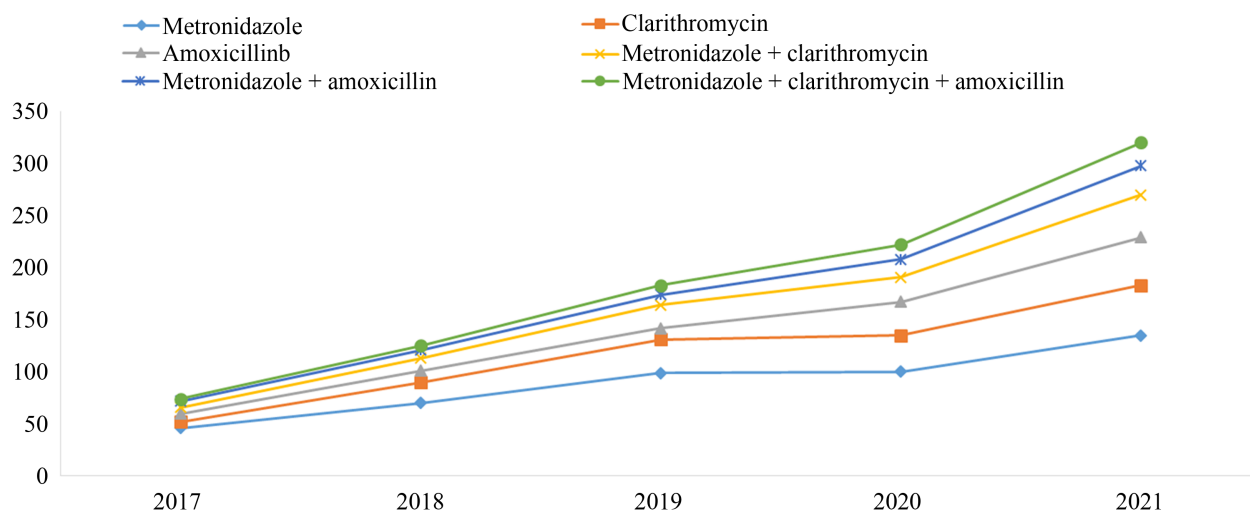
The Hp resistance to metronidazole and clarithromycin in children in Chengdu were: 17.65% (27/153) and 18.06% (78/432) in adults. There were 17.94% of the all strains which were resistant to metronidazole and clarithromycin. The Hp resistance to metronidazole and amoxicillin in children in Chengdu were: 11.76% (18/153) and 13.19% (57/432) in adults. There were 12.82% of the all strains which were resistant to metronidazole and amoxicillin. The Hp resistance to metronidazole, clarithromycin and amoxicillin in children in Chengdu were: 3.92% (6/153) and 10.41% (45/432) in adults. There were 8.71% of the all strains which were resistant to metronidazole, clarithromycin and amoxicillin. There was very significant difference in the rate of resistance to any of the three antibiotics tested when we compared children versus adults (P ≤ 0.05) (Table 1).

### 3.5. The Trend of the Drug Resistance Rate of Hp

The resistance rates of Hp to metronidazole, clarithromycin and amoxicillin were classified as: 76.67% (46/60), 10% (6/60) and 13.33% (8/60) to 77.14% (135/175), 27.42% (48/175) and 26.58% (46/175) from 2017-2021 respectively. The mixed resistance to metronidazole and clarithromycin by Hp was 10% (6/60) to 23.42% (641/175) from 2017 to 2021. The mixed resistance to metronidazole and amoxicillin by Hp was 10% (6/60) to 16.00% (28/175) from 2017 to 2021. The mixed resistance to metronidazole, clarithromycin and amoxicillin by Hp was 3.33% (2/60) to 12.57% (22/175) from 2017 to 2021. In a word, the drug resistance rate of Hp has increased year by year from 2017 to 2021 year, especially the drug resistance rate of Hp to clarithromycin and metronidazole has generally increased (Figure 1 and Table 2).

## 4. DISCUSSION

*Helicobacter pylori* (Hp) is an important cause of chronic gastritis which is closely related to the incidence of peptic ulcer. Not only the eradication of Hp can accelerate the healing of peptic ulcer, but also significantly reduce the recurrence rate of ulcer, so that the ulcer disease is cured. In 1994, the World Health Organization's International Cancer Agency (IARC) officially listed Hp as the first carcinogenic



**Figure 1.** The trend of *H. pylori* strains resistance. Prevalence of *H. pylori* strains resistant to amoxicillin, clarithromycin, and metronidazole isolated in Chengdu City during the period from 2017 to 2021. The numbers of strains studied were 60 in 2017, 90 in 2018, 130 in 2019, 130 in 2020 and 175 in 2021.

**Table 2.** The yearly rate of resistance during the period from 2017 to 2021.

Antibiotic (s)	No. (%) of resistant isolates				
	2017 (n = 60)	2018 (n = 90)	2019 (n = 130)	2020 (n = 130)	2021 (n = 175)
Metronidazole	46 (76.67)	70 (77.78)	99 (76.92)	100 (n = 77.21)	135 (77.14)
Clarithromycin	6 (10)	20 (22.22)	32 (24.61)	35 (26.92)	48 (27.42)
Amoxicillin	8 (13.33)	11 (12.22)	11 (8.46)	32 (24.61)	46 (26.58)
Metronidazole + clarithromycin	6 (10)	12 (13.33)	22 (16.92)	24 (18.46)	41 (23.42)
Metronidazole + amoxicillin	6 (10)	8 (n = 8.89)	10 (7.69)	17 (13.08)	28 (16)
Metronidazole + clarithromycin + amoxicillin	2 (3.33)	4 (4.44)	9 (6.92)	14 (10.77)	22 (12.57)

Resistance to amoxicillin was transient in all cases; it was lost after passage or freezing. Prevalence of *H. pylori* strains resistant to amoxicillin, clarithromycin, and metronidazole isolated in Chengdu City during the period from 2017 to 2021.

factor [1]. Eradication of Hp reduces the recurrence rate after early gastric cancer surgery [1, 2]. Hp infection is closely associated with low-grade malignant gastric mucosa-associated lymphoid tissue (MALT) lymphoma [4, 5]. Eradication of Hp reduces lymphoma and even resolves early low-grade malignant B-cell MALT lymphoma. The treatment of Hp infection has been the most popular topic of the most attention and research by gastrointestinal disease workers, and the biggest problem in the treatment of Hp infection is Hp drug resistance to antibiotics [6], and Hp drug resistance is the main cause of Hp eradication failure [7]. It is severe for the multidrug resistance of Hp.

How to avoid the production of Hp-resistant strains is a key topic in the treatment of Hp infection. Resistance to clarithromycin increased year by year, while resistance to amoxicillin was less frequent. The reported rates of Hp resistance to metronidazole and clarithromycin vary widely everywhere. The widespread use of clarithromycin in children is a major cause for the increase of Hp resistance. Antiantibiotic resistance of Hp tends to increase year by year. Perez et al reported that the primary resistance to metronidazole in Hp in Japan has increased from 6.6% from 1997 to 1998 to 12% clarithromycin from 1999 to 2000 and also from 1999 to 15.2%. In Eastern Europe since 1998, Hp primary resistance to metronidazole, clarithromycin, and amoxicillin reached 37.9%, 9.5%, and 0.9% respectively, and mixed resistance to metronidazole and clarithromycin was 6.1% [9-11]. This study showed that the rate of Hp resistance to metronidazole and clarithromycin was increasing annually in the Chengdu region in China, and also in mixed Hp resistant strains. 17.94% clarithromycin-resistant Hp strains also concurrent metronidazole resistance, suggesting that Hp resistance to clarithromycin is closely related to metronidazole resistance. In many places in China, the drug resistance of *Helicobacter pylori* has increased the trend, the performance is not the same. Chengdu is not the worst but not the best. Compared with the international situation, Chinese drug resistance situation is more serious.

However, a significant increase in amoxicillin resistance was also found. Hp resistance to amoxicillin is increasing year by year, Up to as high as 18.46% in this study. The reasons are as follows: One is the increased attention to *H. pylori*, with or without *H. pylori* infection, any gastrointestinal discomfort is felt caused by *H. pylori* infection and then use triple or quadruple therapy for anti-*H. pylori* therapy; Second, the phenomenon of abusing antibiotics leads to drug resistance under the high pressure of antibiotics; Third, because the people in Sichuan are very hospitable, like to dine out there and increased odds of causing *H. pylori* infection. It also increases the chance of infecting drug resistant strains; the fourth is that the local *H. pylori* may have appeared the variation.

The mechanism of Hp resistance to metronidazole is more complex. It is not very clear, however, most studies believe that mutations in the *rdxA* gene and the *frxA* gene are the main cause of Hp to metronidazole resistance [11-13]. Amoroicillin is one of the drugs most commonly used in clinical practice to treat Hp infection, but Hp resistance to amoxicillin for 20 years is very rare, with a generally reported resistance rate of 0% to 5%. In our study, 18.46% of the isolates were drug-resistant.

Metronidazole has been one of the main drugs in Hp eradication therapy due to its high stability and high activity in the stomach. which is one reason for the rapid increase of Hp to metronidazole resistance [14-16], clarithromycin is considered the most effective antibiotic in the eradication treatment of Hp not only for the eradication treatment of Hp but also in other infectious diseases which has also led to the increased rate of antibiotic resistance to Hp [17-19]. At present, the Hp resistance to metronidazole and clarithromycin in Chengdu area is already a common problem and the resistance rate is increasing year by year. Resistance to amoxicillin also cannot be ignored. We should be paid attention to multiple drug resistance. To avoid the generation of Hp-resistant strains, the indications of Hp eradication should be strictly grasped to avoid antibiotic abuse. The treatment of Hp infection should be regulated. For conditional units, drug sensitivity tests can be done before the treatment to choose sensitive drug treatment.

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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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