**Helicobacter pylori** isolates from ethnic minority patients in Guangxi: Resistance rates, mechanisms, and genotype

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**Abstract**

**AIM:** To investigate the rate of *Helicobacter pylori* (*H. pylori*) resistance to clarithromycin among ethnic minority patients in Guangxi, explore the underlying mechanisms, and analyze factors influencing genotype distribution of *H. pylori* isolates.

**METHODS:** *H. pylori* strains were isolated, cultured and subjected to drug sensitivity testing. The 23S rRNA gene of *H. pylori* isolates was amplified by PCR and analyzed by PCR-RFLP and direct sequencing to detect point mutations. REP-PCR was used for genotyping of *H. pylori* isolates, and NTsys_2 software was used for clustering analysis based on REP-PCR DNA fingerprints. Factors potentially influencing genotype distribution of *H. pylori* isolates were analyzed.

**RESULTS:** The rate of clarithromycin resistance was 31.3%. A2143G and A2144G mutations were detected in the 23S rRNA gene of all clarithromycin-resistant *H. pylori* isolates. At a genetic distance of 78%, clarithromycin-resistant *H. pylori* isolates could be divided into six groups. Significant clustering was noted among *H. pylori* isolates from patients with peptic ulcer or gastritis.

**CONCLUSION:** The rate of clarithromycin resistance is relatively high in ethnic minority patients in Guangxi. Main mechanisms of clarithromycin resistance are A2143G and A2144G mutations in the 23S rRNA gene of all clarithromycin-resistant *H. pylori* isolates. At a genetic distance of 78%, clarithromycin-resistant *H. pylori* isolates could be divided into six groups. Significant clustering was noted among *H. pylori* isolates from patients with peptic ulcer or gastritis.
mycin in this region may be caused by the long-term and/or wide use of clarithromycin, which can decrease the populations of sensitive bacteria and promote the propagation of drug-resistant bacteria. Of note, this study also found that there existed multidrug resistant \textit{H. pylori} strains (resistant to amoxicillin, metronidazole, tetracycline, and levofloxacin).

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**INTRODUCTION**

\textit{Helicobacter pylori} (\textit{H. pylori}) is a frequent cause of human chronic gastritis and peptic ulcer, and is also involved in the pathogenesis of gastric cancer and mucosa-associated lymphoid tissue lymphoma\cite{1-4}. The wide application of antibiotics in \textit{H. pylori} eradication therapy has led to the increasing prevalence of \textit{H. pylori} resistance to antibiotics. Antibiotic resistance is a major cause of treatment failure. Due to the differences in doctors’ prescribing habits, patients’ medical history, and diets, the rates of \textit{H. pylori} infection and antibiotic resistance vary among different countries or regions\cite{5-10}. Therefore, monitoring and research of antibiotic-resistant \textit{H. pylori} strains from different regions can help not only understand the status of antibiotic resistance and guide clinical medication, but also overcome antibiotic resistance, increase the rate of eradication and avoid the emergence of drug-resistant strains. Guangxi is home to many ethnic minorities besides the Han Chinese, including Zhuang, Yao and Miao. Particularly, Zhuang has the largest population. These ethnic minorities have distinct living and eating habits. Additionally, the level of economic development and the standard of living are relatively low in this region, and there are fewer types of antibiotics available. Therefore, it is possible that the rates of \textit{H. pylori} infection and antibiotic resistance in this region are significantly different from those in other regions of China. In the present study, we investigated the rate of \textit{H. pylori} resistance to clarithromycin among ethnic minority patients in this region, explored the mechanism of clarithromycin resistance, and analyzed factors potentially influencing clarithromycin resistance, with an aim to reduce the rate of \textit{H. pylori} resistance to antibiotics and improve the effect of treatment.

**MATERIALS AND METHODS**

**\textit{H. pylori} strains**

Between May 2011 and May 2012, 164 gastric mucosal biopsies were collected from patients with gastritis or peptic ulcer at the Department of Gastroscopy, the Affiliated Hospital of Youjiang Medical University for Nationalities. The samples were inoculated into Columbia medium and cultured for 5-7 d in a microaerobic bag at 37 ℃. The suspected strains were confirmed as \textit{H. pylori} by Gram-staining and urease, oxidase and catalase tests.

**Drug sensitivity testing**

The bacterial suspension was adjusted to a density of \(1.0 \times 10^8\) CFU/mL and plated on the Columbia sheep blood agar. After the clarithromycin discs were plated, the plates were incubated at 37 ℃ for 5 d. The diameter of inhibition zone was measured. According to the criteria recommended by the 2012 Clinical and Laboratory Standards Institute (CLSI), strains were considered sensitive to clarithromycin when the diameter of inhibition zone was \(\geq 17\) mm and resistant to clarithromycin when the diameter was \(\leq 13\) mm.

**\textit{H. pylori} DNA extraction and PCR amplification**

Genomic DNA was isolated from \textit{H. pylori} cells using a commercial kit (General Biotech, Shanghai, China) according to the manufacturer's instructions. The A2144G and A2143G loci were amplified by PCR in a 50-μL reaction system consisting of 29.5 μL ddH2O, 6.3 μL 10 × PCR buffer, 5.0 μL dNTPs (25 mmol/L), 0.5 μL Taq polymerase (5 kU/L), 5.7 μL of each forward and reverse primer (10 μmol/L), and 1.3 μL DNA template. The primers were designed as previously described\cite{5} and their sequences were 5′-CCA CAG CGA TGT GGT AGCCC-3′ (reverse), which yields a fragment of 425 bp. PCR cycling parameters were pre-denaturation at 94.0 ℃ for 4 min, 32 cycles of denaturation at 94.0 ℃ for 40 s, annealing at 61.5 ℃ for 1 min, and extension at 72.0 ℃ for 1 min, and a final extension at 72.0 ℃ for 7 min. PCR products were resolved by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining under ultraviolet light.

**PCR-RFLP**

The two most common mutations associated with clarithromycin resistance (A2143G and A2144G) result in the generation of two new restriction sites for \textit{Bbs} I and \textit{Bsa} I. To examine whether these two mutations were present, the above PCR products (8 μL) were incubated with \textit{Bbs} I at 37 ℃ for 24 h or with \textit{Bsa} I at 50 ℃ for 24 h. After enzyme digestion, the reaction products (10 μL) were resolved by 1% agarose gel electrophoresis and visualized by ethidium bromide staining under ultraviolet light.

**DNA sequencing**

PCR products for 1 sensitive strain and 5 clarithromycin-resistant strains were randomly selected for DNA sequencing. DNA sequencing was performed by General Biotech (Shanghai, China). DNA Tool 6.0 program was used to analyze the 23S rRNA gene sequences of...
clarithromycin-sensitive and -resistant strains, and the sequences were compared with that of HPJ99 strain (NC-000921) deposited in the genome database (National Center for Biotechnology Information, NCBI).

**REP-PCR**

REP-PCR was performed in a 25-μL reaction system consisting of 14.25 μL ddH2O, 2.5 μL 10 × PCR buffer, 0.5 μL dNTPs, 0.5 μL Taq polymerase (2 U), 0.5 μL of each forward and reverse primer, 0.5 μL MgCl2 and 5 μL DNA template. The primers were designed as previously described and their sequences were 5′-CGGIC-TCIGcIIII-3′ (forward) and 5′-ICGICITFATCIGGCCTAC-3′ (reverse), where I represents inosine. PCR cycling parameters were pre-denaturation at 95.0℃ for 30 s, 80℃ for 2 min, 65 cycles of denaturation at 95.0℃ for 30 s, annealing at 40℃ for 1 min, and extension at 65.0℃ for 8 min. PCR products were resolved by 1.0% agarose gel electrophoresis and visualized by ethidium bromide staining under ultraviolet light.

**Clustering analysis**

Based on REP-PCR DNA fingerprints, the band was scored 1 if there was a mobility shift and 0 if there was not. NTsys_2 software was used for clustering analysis.

**Statistical analysis**

The rate of clarithromycin resistance is expressed as a percentage (%).

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**RESULTS**

**Bacterial isolation**

A total of 115 clinical isolates were identified as *H. pylori*, of which 82 were isolated from patients with peptic ulcer and 33 from patients with gastritis.

**Rate of clarithromycin resistance**

Drug sensitivity testing revealed that there were 36 *H. pylori* strains that were resistant to clarithromycin, and the rate of clarithromycin resistance was 31.3% (36/115).

**PCR-RFLP analysis of the 23S rRNA gene**

Ten each of clarithromycin-sensitive and -resistant strains were randomly selected for PCR-RFLP analysis of the 23S rRNA gene. A 425-bp fragment of interest was amplified in all clarithromycin-sensitive and -resistant strains (Figure 1).

**Clarithromycin resistance-associated mutations A2143G and A2144G** result in the generation of two new restriction sites for *Bbs* I and *Bsa* I (Figure 2). Although the 425-bp fragment could be amplified from all *H. pylori* strains, only the fragment from the 10 clarithromycin-resistant strains could be digested by *Bbs* I and *Bsa* I, and the digestion resulted in the generation of two bands (319 bp and 106 bp for *Bsa* I digestion, and 331 bp and 94 bp for *Bbs* I digestion, Figure 3). This finding suggests the presence of A2143G and A2144G mutations in clarithromycin-resistant strains. In contrast, the fragment from the 10 clarithromycin-sensitive strains could not be digested by *Bbs* I and *Bsa* I, indicating the absence of A2143G and A2144G mutations in clarithromycin-sensitive strains (Figure 3). Of note, the 425-bp fragment could not be completely digested by both enzymes in all clarithromycin-resistant strains.

**DNA sequencing**

PCR products for 1 sensitive strain and 5 clarithromycin-resistant strains were randomly selected for DNA sequencing. The obtained DNA sequences were compared with that of HPJ99 strain deposited in the NCBI genome database. The results confirmed the presence of A2143G and A2144G mutations in clarithromycin-resistant strains,
Clustering analysis

The NTsys-2 software was used to analyze the similarity among the randomly selected clarithromycin-resistant and -sensitive H. pylori isolates. At a genetic distance of 78%, 26 clarithromycin-resistant H. pylori isolates could be classified into 6 genotypes (Figure 6), and 26 clarithromycin-sensitive H. pylori isolates could be classified into 4 genotypes (Figure 7).

REP-PCR analysis

Twenty-six each of randomly selected clarithromycin-resistant and -sensitive H. pylori isolates and the standard strain ATCC43504 were subjected to REP-PCR analysis, and the results are shown in Tables 1 and 2 and Figure 5.

Table 1  Clinical characteristics of 26 patients from whom clarithromycin-resistant Helicobacter pylori strains were isolated

| No. | Strain   | Gender | Age | Ethnicity | Region | Disease type | History of medication | Family history of gastric disease | Antibiotic resistance |
|-----|----------|--------|-----|-----------|--------|--------------|----------------------|-----------------------------------|----------------------|
| 1   | GXHP039  | F      | 46  | H         | LY     | PU           | +                    | +                                 | CA                   |
| 2   | GXHP051  | F      | 46  | H         | BS     | PU           | +                    | +                                 | CA                   |
| 3   | GXHP071  | F      | 39  | Ch        | TY     | CG           | -                    | -                                 | C                    |
| 4   | GXHP080  | F      | 54  | Ch        | BS     | CG           | +                    | -                                 | CAS                  |
| 5   | GXHP043  | F      | 47  | H         | BS     | PU           | +                    | -                                 | CA                   |
| 6   | GXHP093  | M      | 46  | Ch        | TL     | CG           | -                    | -                                 | CASG                 |
| 7   | GXHP004  | F      | 49  | Ch        | TY     | PU           | -                    | -                                 | C                    |
| 8   | GXHP014  | F      | 46  | Ch        | BS     | PU           | +                    | -                                 | CAS                  |
| 9   | GXHP016  | F      | 57  | Ch        | BS     | PU           | +                    | +                                 | CASG                 |
| 10  | GXHP019  | M      | 52  | Ch        | LY     | PU           | -                    | +                                 | C                    |
| 11  | GXHP021  | M      | 51  | Ch        | TL     | PU           | +                    | +                                 | CASG                 |
| 12  | GXHP023  | M      | 48  | Ch        | LY     | PU           | +                    | -                                 | CAG                  |
| 13  | GXHP026  | M      | 41  | Ch        | TY     | PU           | -                    | -                                 | C                    |
| 14  | GXHP021  | M      | 46  | H         | TY     | CG           | +                    | -                                 | C                    |
| 15  | GXHP085  | M      | 53  | Ch        | TY     | CG           | +                    | +                                 | C                    |
| 16  | GXHP034  | M      | 51  | Ch        | BS     | PU           | -                    | +                                 | CAG                  |
| 17  | GXHP090  | M      | 41  | Ch        | BS     | CG           | -                    | -                                 | C                    |
| 18  | GXHP067  | M      | 41  | H         | BS     | PU           | +                    | -                                 | C                    |
| 19  | GXHP056  | M      | 39  | H         | TY     | PU           | -                    | +                                 | CA                   |
| 20  | GXHP100  | F      | 47  | H         | LY     | CG           | -                    | -                                 | CA                   |
| 21  | GXHP104  | F      | 46  | H         | TY     | CG           | +                    | -                                 | CG                   |
| 22  | GXHP107  | F      | 56  | H         | BS     | CG           | -                    | +                                 | CAS                  |
| 23  | GXHP110  | F      | 59  | H         | BS     | CG           | +                    | +                                 | CAG                  |
| 24  | GXHP113  | M      | 46  | H         | TL     | CG           | -                    | +                                 | C                    |
| 25  | GXHP115  | M      | 53  | H         | LY     | CG           | -                    | +                                 | CAG                  |
| 26  | GXHP116  | M      | 46  | H         | BS     | CG           | +                    | +                                 | C                    |

Table 2  REP-PCR analysis of clarithromycin-resistant H. pylori isolates from ethnic minority patients in Guangxi

| No. | Strain   | Gender | Age | Ethnicity | Region | Disease type | History of medication | Family history of gastric disease | Antibiotic resistance |
|-----|----------|--------|-----|-----------|--------|--------------|----------------------|-----------------------------------|----------------------|
| 1   | GXHP039  | F      | 46  | H         | LY     | PU           | +                    | +                                 | CA                   |
| 2   | GXHP051  | F      | 46  | H         | BS     | PU           | +                    | +                                 | CA                   |
| 3   | GXHP071  | F      | 39  | Ch        | TY     | CG           | -                    | -                                 | C                    |
| 4   | GXHP080  | F      | 54  | Ch        | BS     | CG           | +                    | -                                 | CAS                  |
| 5   | GXHP043  | F      | 47  | H         | BS     | PU           | +                    | -                                 | CA                   |
| 6   | GXHP093  | M      | 46  | Ch        | TL     | CG           | -                    | -                                 | CASG                 |
| 7   | GXHP004  | F      | 49  | Ch        | TY     | PU           | -                    | -                                 | C                    |
| 8   | GXHP014  | F      | 46  | Ch        | BS     | PU           | +                    | -                                 | CAS                  |
| 9   | GXHP016  | F      | 57  | Ch        | BS     | PU           | +                    | +                                 | CASG                 |
| 10  | GXHP019  | M      | 52  | Ch        | LY     | PU           | -                    | +                                 | C                    |
| 11  | GXHP021  | M      | 51  | Ch        | TL     | PU           | +                    | +                                 | CASG                 |
| 12  | GXHP023  | M      | 48  | Ch        | LY     | PU           | +                    | -                                 | CAG                  |
| 13  | GXHP026  | M      | 41  | Ch        | TY     | PU           | -                    | -                                 | C                    |
| 14  | GXHP021  | M      | 46  | H         | TY     | CG           | +                    | -                                 | C                    |
| 15  | GXHP085  | M      | 53  | Ch        | TY     | CG           | +                    | +                                 | C                    |
| 16  | GXHP034  | M      | 51  | Ch        | BS     | PU           | -                    | +                                 | CAG                  |
| 17  | GXHP090  | M      | 41  | Ch        | BS     | CG           | -                    | -                                 | C                    |
| 18  | GXHP067  | M      | 41  | H         | BS     | PU           | +                    | -                                 | C                    |
| 19  | GXHP056  | M      | 39  | H         | TY     | PU           | -                    | +                                 | CA                   |
| 20  | GXHP100  | F      | 47  | H         | LY     | CG           | -                    | -                                 | CA                   |
| 21  | GXHP104  | F      | 46  | H         | TY     | CG           | +                    | -                                 | CG                   |
| 22  | GXHP107  | F      | 56  | H         | BS     | CG           | -                    | +                                 | CAS                  |
| 23  | GXHP110  | F      | 59  | H         | BS     | CG           | +                    | +                                 | CAG                  |
| 24  | GXHP113  | M      | 46  | H         | TL     | CG           | -                    | +                                 | C                    |
| 25  | GXHP115  | M      | 53  | H         | LY     | CG           | -                    | +                                 | CAG                  |
| 26  | GXHP116  | M      | 46  | H         | BS     | CG           | +                    | +                                 | C                    |

PU: Peptic ulcer; CG: Gastritis; Ch: Chuang; H: Han; M: Male; F: Female; BS: Baise; TY: Tianyang; LY: Lingyu; TL: Tianlin; +: A clear history; -: An unclear history; C: Clarithromycin; G: Gentamycin; S: Streptomycin; A: Amoxicillin.

but not in clarithromycin-sensitive strains (Figure 4).
Factors influencing genotype distribution of H. pylori isolates

Factors influencing genotype distribution of H. pylori isolates may include disease type, ethnicity, gender, age, region, history of antibiotic medication, history of gastric diseases, and multidrug resistance. The frequencies of the presence of these factors in the patients are presented in Tables 3 and 4.

DISCUSSION

Rate of H. pylori resistance to clarithromycin in ethnic minority patients in Guangxi

Antibiotic resistance is a main factor affecting therapeutic effects in patients with H. pylori infection. Particularly, the rates of H. pylori resistance to clarithromycin and metronidazole have been increasing year by year, and accordingly, the rate of H. pylori eradication achieved with regimens containing either of the two antibiotics becomes lower and lower. The rate of H. pylori resistance to antibiotics varies among different countries or regions, although it shows an upward trend[11-15]. From 1996 to 2004 in Japan, the prevalence of H. pylori resistance to clarithromycin has increased to 30%[16]. In Vietnam in 2008[17], the rate of clarithromycin resistance was 33%, and the rate in Ho Chi Minh City (49%) was obviously higher than that in Hanoi (18.5%). In China, the rate of H. pylori resistance to antibiotics varies among different regions. For example, a study on H. pylori resistance to antibiotics in Beijing, Shanghai and Wenzhou[18] showed that the rate of resistance to metronidazole was highest, followed by clarithromycin. In 2009 in Dongguan, the rate of H. pylori resistance to clarithromycin was 27.6%.[19]

The present study showed that the rate of H. pylori resistance to clarithromycin was 31.3% in ethnic minority patients in Guangxi, slightly higher than that in Dongguan in 2009 but significantly higher than the reported resistance rate in 2008 in the same region (Guangxi).
significant increase in the rate of *H. pylori* resistance to clarithromycin in this region may be caused by the long-term and/or wide use of clarithromycin, which can decrease the populations of sensitive bacteria and promote the propagation of drug-resistant bacteria. Of note, this study also found that there existed multidrug resistant *H. pylori* strains (resistant to amoxicillin, metronidazole, tetracycline, and levofloxacin). Therefore, we recommend that, in order to improve the rate of *H. pylori* eradication, tests for *H. pylori* antibiotic resistance should be

| No. | Strain |
|-----|--------|
| 1   | GXHP069 M 46 Ch TL CG + + |
| 2   | GXHP070 M 43 Ch LY CG + + |
| 3   | GXHP096 F 36 Ch BS CG - + |
| 4   | GXHP119 F 48 H TY CG - - |
| 5   | GXHP094 F 46 Ch BS CG + - |
| 6   | GXHP74 M 47 Ch TL CG + - |
| 7   | GXHP116 F 46 H BS CG - + |
| 8   | GXHP075 M 45 Ch LY CG - - |
| 9   | GXHP076 M 48 Ch TY CG - + |
| 10  | GXHP079 M 53 Ch TL CG + + |
| 11  | GXHP078 M 52 Ch BS CG + + |
| 12  | GXHP101 M 42 H BS CG - + |
| 13  | GXHP102 M 56 H BS CG + - |
| 14  | GXHP005 M 35 Ch BS PU - + |
| 15  | GXHP006 M 41 Ch TL PU - + |
| 16  | GXHP007 M 60 Ch TL PU + + |
| 17  | GXHP008 M 38 Ch BS PU - - |
| 18  | GXHP024 F 50 Ch TL PU + + |
| 19  | GXHP025 F 39 Ch BS PU - - |
| 20  | GXHP027 F 59 Ch BS PU - - |
| 21  | GXHP140 M 51 H BS PU + + |
| 22  | GXHP041 M 52 H BS PU + + |
| 23  | GXHP052 F 53 H BS PU - - |
| 24  | GXHP053 F 47 H BS PU + + |
| 25  | GXHP054 F 49 H BS PU - - |
| 26  | GXHP050 M 72 H LY PU - + |
established in this region, sensitive antibiotics should be selected based on antibiotic sensitivity of *H. pylori* strains, and the research on the mechanisms of antibiotic resistance should be enhanced.

**Mechanisms of *H. pylori* resistance to clarithromycin in ethnic minority patients in Guangxi**

Clarithromycin, as a new generation of macrolide antibiotic, is acid-stable and can be dissolved in gastric

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**Table 3** Frequencies of the presence of factors influencing genotype distribution of clarithromycin-resistant strains

| CG  | PU | Ch | H  | M  | F  | TY | TL | LY | BS | HMR | HFHD | 30-40 yr | 41-50 yr | 51-60 yr |
|-----|----|----|----|----|----|----|----|----|----|-----|-------|---------|---------|---------|
| Group I | 0  | 2  | 0  | 2  | 2  | 0  | 0  | 0  | 1  | 1  | 2  | 2  | 0  | 0  | 2  | 0  |
| Group II | 0  | 11 | 8  | 3  | 4  | 7  | 3  | 1  | 2  | 5  | 4  | 4  | 4  | 1  | 6  | 4  |
| Group III | 1  | 0  | 0  | 1  | 1  | 0  | 0  | 0  | 1  | 1  | 1  | 1  | 0  | 0  | 1  | 0  |
| Group IV | 8  | 0  | 2  | 6  | 5  | 3  | 2  | 1  | 1  | 4  | 4  | 4  | 5  | 1  | 3  | 4  |
| Group V  | 3  | 0  | 2  | 1  | 0  | 3  | 2  | 0  | 0  | 1  | 1  | 1  | 1  | 0  | 2  | 1  |
| Group VI | 1  | 0  | 1  | 0  | 0  | 1  | 0  | 1  | 0  | 1  | 0  | 0  | 0  | 1  | 0  | 0  |

CG: Gastritis; PU: Peptic ulcer; Ch: Chuang; H: Han; M: Male; F: Female; TY: Tianyang; TL: Tianlin; LY: Lingyu; BS: Baise; HMR: History of multidrug resistance; HM: History of medication; HFGD: Family history of gastric disease.
juice that has a low pH level. Therefore, it has good oral bioavailability and its concentration is high in the gastric mucosa. Additionally, clarithromycin has few adverse reactions. Since clarithromycin monotherapy can achieve an eradication rate between 42%-54%, it is one of the currently known antibiotics that have the strongest effect on H. pylori. Compared with triple therapy without clarithromycin, clarithromycin-containing triple therapy can increase the rate of H. pylori eradication by 10%-20%. Therefore, clarithromycin is the main antibiotic used in regimens for H. pylori. However, significant resistance of H. pylori to clarithromycin has been observed, and the rate of clarithromycin resistance has increased, especially after an initial failure.[21-23] Clarithromycin exerts antibacterial effects by penetrating the bacteria cell wall, binding to the domain V of the 23S ribosomal RNA of the 50S subunit of the bacterial ribosome, inhibiting peptidyl transferase activity, interfering with amino acid translocation and thereby suppressing bacterial protein synthesis. With regard to the mechanisms of H. pylori resistance to clarithromycin, the consensus view is point mutations in the 23S rRNA gene in different regions and different ethnicity groups. Many studies have shown that point mutations in the 23S rRNA gene in H. pylori strains can vary among patients and among different disease types, and the results showed that strains isolated from different patients had different DNA fingerprints (Figure 5A).

**Genotype variation in H. pylori isolates in Guangxi and factors influencing genotype distribution**

To investigate the association of genotypes of H. pylori isolates from ethnic minority patients in Guangxi with disease type, ethnicity and multidrug resistance, REP-PCR was used to genotype clarithromycin-resistant H. pylori isolates from patients of different ethnicity or with different disease types, and the results showed that strains isolated from different patients had different DNA fingerprints (Figure 5A).

The NTsys_2 software was used to perform clustering analysis. The dendrogram of REP-PCR DNA fingerprints for clarithromycin-resistant H. pylori strains (Figure 6A) showed a similarity of 100% among GXHP014, GXHP016, GXHP019, GXHP021, GXHP023 and GXHP026; between GXHP014 and GXHP107; and between GXHP110 and GXHP113. These three groups shared a similarity of 90%. In addition, GXHP014 and GXHP107 had a similarity of 95.2% to GXHP110 and GXHP113. At a genetic distance of 78%, the 26 strains of clarithromycin-resistant H. pylori were divided into 6 groups, which are as follows: (1) group I: This group includes GXHP039 and GXHP043, which were isolated from a woman with peptic ulcer in Baise and a woman with the same disease in Lingyun, respectively. The two women, ranging in age between 41 and 50 years old, had a history of clarithromycin use, but their family history of gastric disease was unknown. The two strains were also resistant to amoxicillin, streptomycin, and gentamicin; (2) group II: This group includes GXHP051, GXHP034, GXHP004, GXHP014, GXHP019, GXHP021, GXHP023, GXHP026, GXHP056 and GXHP067. All the 11 strains were isolated from patients with peptic ulcer. GXHP034, GXHP004, GXHP014, GXHP016, GXHP019, GXHP021, GXHP023, GXHP026 and GXHP056 came from 9 Zhuang patients (6 females and 3 males) in Baise, Tianlin, Lingyun, or Tianyang. They had an unknown history of medication or a family history of gastric diseases. GXHP014, GXHP019, GXHP021 and GXHP023, GXHP026 and GXHP056 came from 9 Zhuang patients (6 females and 3 males) in Baise, Tianlin, Lingyun, or Tianyang. They had an unknown history of medication or a family history of gastric diseases. GXHP014, GXHP019, GXHP021 and GXHP023, GXHP026 and GXHP056 came from 9 Zhuang patients (6 females and 3 males) in Baise, Tianlin, Lingyun, or Tianyang. They had an unknown history of medication or a family history of gastric diseases.

**Table 4** Frequencies of the presence of factors influencing genotype distribution of clarithromycin-sensitive strains

| Group | CG | PU | Ch | H | M | F | TY | TL | LY | BS | HM | FHGD | 30-40 yr | 41-50 yr | 51-60 yr |
|-------|----|----|----|---|---|---|----|----|----|----|----|--------|----------|----------|----------|
| I     | 9  | 0  | 7  | 2 | 0 | 9 | 1  | 2  | 2  | 4  | 2  | 6      | 0        | 6        | 3        |
| II    | 4  | 0  | 2  | 2 | 4 | 0 | 1  | 0  | 0  | 3  | 1  | 3      | 1        | 3        | 0        |
| III   | 0  | 11 | 5  | 6 | 4 | 7 | 1  | 2  | 1  | 7  | 3  | 7      | 3        | 3        | 5        |
| IV    | 0  | 2  | 1  | 1 | 2 | 0 | 1  | 0  | 0  | 1  | 0  | 1      | 1        | 1        | 0        |

CG: Gastritis; PU: Peptic ulcer; Ch: Chuang; H: Han; M: Male; F: Female; TY: Tianyang; TL: Tianlin; LY: Lingyu; BS: Baise; HM: History of medication; FHGD: Family history of gastric disease.
tamicin. In this group, one patient was in the 30-40 age group, 6 in the 41-50 age group, and 4 in the 51-60 age group; (3) group III: This group includes only GXHP100. This strain came from a Han male with chronic gastritis in Lingyun. He had a history of clarithromycin use and family history of gastric disease. The strain was also resistant to amoxicillin.

4. Helicobacter pylori-related diseases.

- H. pylori is a frequent cause of human chronic gastritis and is involved in the pathogenesis of gastric cancer and mucosa-associated lymphoid tissue lymphoma. The wide application of antibiotics has led to the increasing prevalence of H. pylori eradication therapy. Antibiotic resistance is a major cause of treatment failure.

5. Antibiotic resistance.

- Antibiotic resistance is a main factor affecting therapeutic effects in patients with H. pylori infection. Particularly, the rates of H. pylori resistance to clarithromycin and metronidazole have been increasing year by year, and accordingly, the rate of H. pylori eradication achieved with regimens containing either of the two antibiotics becomes lower and lower.

6. Research frontiers.

- The rate of clarithromycin resistance is relatively high in ethnic minority patients in Guangxi. The mechanism of clarithromycin resistance is A2143G and A2144G mutations in the 23S rRNA gene. Clarithromycin-resistant H. pylori isolates can be divided into six groups based on REP-PCR DNA fingerprints.

7. COMMENTS.

- The manuscript is very interesting. The authors try to investigate the rate of H. pylori resistance to clarithromycin in ethnic minority patients in Guangxi, which may help design the treatment for gastric diseases. The data are very interesting. The background is well designed. The data are very interesting. The research is well designed. The data are very interesting.

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April 28, 2014 | Volume 20 | Issue 16 | 4769
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