

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

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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. Clarithromycin-resistant *H. pylori* isolates can be divided into six groups based on REP-PCR DNA fingerprints. Several factors such as disease type may influence the genotype distribution of *H. pylori* isolates.

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Key words: *Helicobacter pylori*; Antibiotic resistance; Mechanism; Clarithromycin; Genotype

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

Core tip: The present study showed that the rate of *Helicobacter pylori* (*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). The significant increase in the rate of *H. pylori* resistance to clarithro-

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 *H. pylori* strains (resistant to amoxicillin, metronidazole, tetracycline, and levofloxacin).

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INTRODUCTION

Helicobacter pylori (*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^[1-4]. The wide application of antibiotics in *H. pylori* eradication therapy has led to the increasing prevalence of *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 *H. pylori* infection and antibiotic resistance vary among different countries or regions^[5-10]. Therefore, monitoring and research of antibiotic-resistant *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 *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 *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 *H. pylori* resistance to antibiotics and improve the effect of treatment.

MATERIALS AND METHODS

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 °C. The suspected strains were confirmed as *H. pylori* by Gram-staining and urease, oxidase and catalase tests.

Drug sensitivity testing

The bacterial suspension was adjusted to a density of 1.0×10^8 CFU/mL and plated on the Columbia sheep blood agar. After the clarithromycin discs were plated, the plates were incubated at 37 °C 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 ≥ 17 mm and resistant to clarithromycin when the diameter was ≤ 13 mm.

H. pylori DNA extraction and PCR amplification

Genomic DNA was isolated from *H. pylori* cells using a commercial kit (Generay Biotech, Shanghai, China) according to the manufacturer's instructions. The A2144G and A2143G loci were amplified by PCR in a 50- μ L reaction system consisting of 29.5 μ L ddH₂O, 6.3 μ L $10 \times$ PCR buffer, 5.0 μ L dNTPs (25 mmol/L), 0.5 μ L Taq polymerase (5 kU/L), 3.7 μ L of each forward and reverse primer (10 μ mol/L), and 1.3 μ L DNA template. The primers were designed as previously described^[5] and their sequences were 5'-CCA CAG CGA TGT GGT CTCAG-3' (forward) and 5'-CTC CAT AAG AGC CAA AGCCC-3' (reverse), which yields a fragment of 425 bp. PCR cycling parameters were pre-denaturation at 94.0 °C for 4 min, 32 cycles of denaturation at 94.0 °C for 40 s, annealing at 61.5 °C for 1 min, and extension at 72.0 °C for 1 min, and a final extension at 72.0 °C 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 *Bhs* I and *Bsa* I. To examine whether these two mutations were present, the above PCR products (8 μ L) were incubated with *Bhs* I at 37 °C for 24 h or with *Bsa* I at 50 °C for 24 h. After enzyme digestion, the reaction products (10 μ L) 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 Generay Biotech (Shanghai, China). DNATool 6.0 program was used to analyze the 23S rRNA gene sequences of

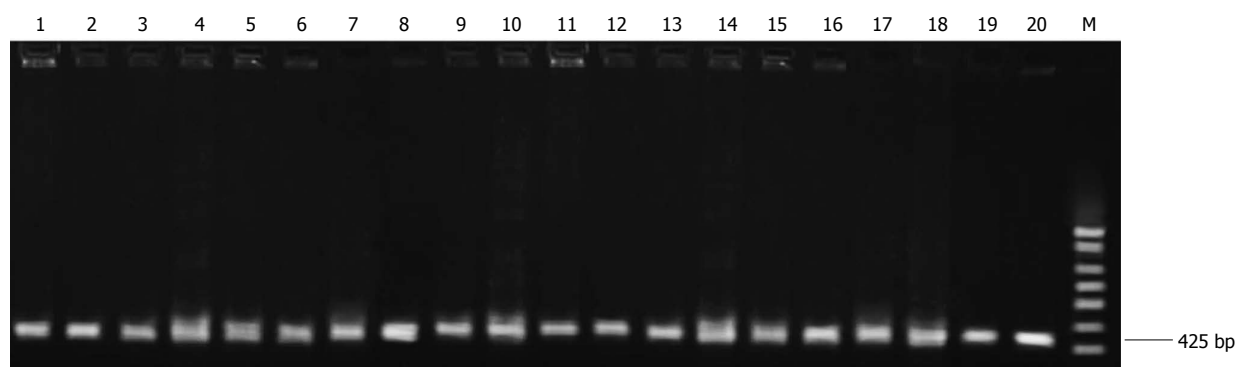


Figure 1 PCR amplification of the 23S rRNA gene of *Helicobacter pylori* strains. 1-10: Randomly selected clarithromycin-sensitive strains; 11-20: Randomly selected clarithromycin-resistant strains; M: 100 bp DNA ladder.

WT 5'-GCA AGA CGG AAA GAC CCC GTG GAC-3'

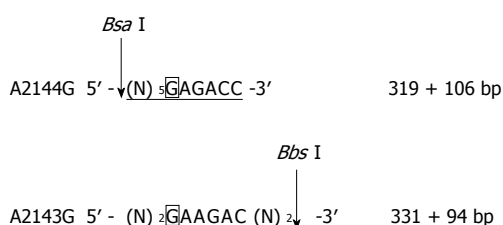


Figure 2 Two new restriction sites for *Bbs* I and *Bsa* I caused by clarithromycin resistance-associated mutations A2143G and A2144G in the 23S rRNA gene of *Helicobacter pylori* strains.

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- μ L reaction system consisting of 14.25 μ L ddH₂O, 2.5 μ L 10 \times PCR buffer, 0.5 μ L dNTPs, 0.5 μ L Taq polymerase (2 U), 0.5 μ L of each forward and reverse primer, 0.25 μ L MgCl₂ and 5 μ L DNA template. The primers were designed as previously described^[6] and their sequences were 5'-CGGIC-TAcIGCIGcIII-3' (forward) and 5'-ICGICITFATCIG-GCCTAC-3' (reverse), where I represents inosine. PCR cycling parameters were pre-denaturation at 95.0 $^{\circ}$ C for 30 s, 80 $^{\circ}$ C for 2 min, 65 cycles of denaturation at 95.0 $^{\circ}$ C for 30 s, annealing at 40 $^{\circ}$ C for 1 min, and extension at 65.0 $^{\circ}$ C for 8 min, and a final extension at 65.0 $^{\circ}$ C 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 (%).

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,

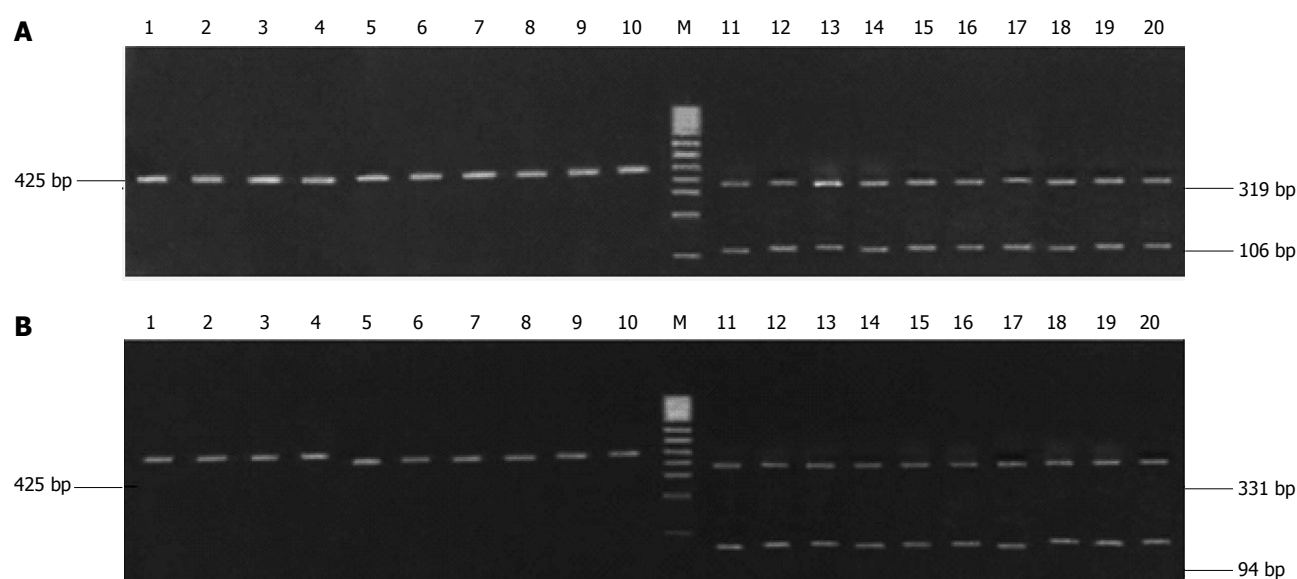


Figure 3 Digestion of the amplified fragment of the 23S rRNA gene from *Helicobacter pylori* isolates with restriction endonuclease *Bbs* I (A) and *Bsa* I (B). 1-10: Randomly selected clarithromycin-sensitive strains; 11-20: Randomly selected clarithromycin-resistant strains; M: 100 bp DNA ladder.

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	GXHP121	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	GXHP120	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).

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.

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

J99 1428504	CCAAAAACACAGCACTTTGCCAACTCGTAAGAGGAAGTATAAGGTGTGACGCCTGCCCGG
S 1	CCAAAAACACAGCACTTTGCCAACTCGTAAGAGGAAGTATAAGGTGTGACGCCTGCCCGG
R1 1	CCAAAAACACAGCACTTTGCCAACTCGTAAGAGGAAGTATAAGGTGTGACGCCTGCCCGG
R2 1	CCAAAAACACAGCACTTTGCCAACTCGTAAGAGGAAGTATAAGGTGTGACGCCTGCCCGG
R3 1	CCAAAAACACAGCACTTTGCCAACTCGTAAGAGGAAGTATAAGGTGTGACGCCTGCCCGG
R4 1	CCAAAAACACAGCACTTTGCCAACTCGTAAGAGGAAGTATAAGGTGTGACGCCTGCCCGG
R5 1	CCAAAAACACAGCACTTTGCCAACTCGTAAGAGGAAGTATAAGGTGTGACGCCTGCCCGG
J99 1428324	GACCTGCATGAATGGCGTAACGAGATGGGAGCTGTCTCAACCAGAGATTCAGTGAAATTG
S 81	GACCTGCATGAATGGCGTAACGAGATGGGAGCTGTCTCAACCAGAGATTCAGTGAAATTG
R1 81	GACCTGCATGAATGGCGTAACGAGATGGGAGCTGTCTCAACCAGAGATTCAGTGAAATTG
R2 81	GACCTGCATGAATGGCGTAACGAGATGGGAGCTGTCTCAACCAGAGATTCAGTGAAATTG
R3 81	GACCTGCATGAATGGCGTAACGAGATGGGAGCTGTCTCAACCAGAGATTCAGTGAAATTG
R4 81	GACCTGCATGAATGGCGTAACGAGATGGGAGCTGTCTCAACCAGAGATTCAGTGAAATTG
R5 81	GACCTGCATGAATGGCGTAACGAGATGGGAGCTGTCTCAACCAGAGATTCAGTGAAATTG
J99 1428264	TAGTGGAGGTGAAAAATTCCTCCTACCCGCGGCAAGACGGAAAGACCCCGTGACCTTTAC
S 241	TAGTGGAGGTGAAAAATTCCTCCTACCCGCGGCAAGACGGAAAGACCCCGTGACCTTTAC
	A2143G, A2144G
	↓
R1 241	TAGTGGAGGTGAAAAATTCCTCCTACCCGCGGCAAGACGGAGGGACCCCGTGACCTTTAC
R2 241	TAGTGGAGGTGAAAAATTCCTCCTACCCGCGGCAAGACGGAGGGACCCCGTGACCTTTAC
R3 241	TAGTGGAGGTGAAAAATTCCTCCTACCCGCGGCAAGACGGAGGGACCCCGTGACCTTTAC
R4 241	TAGTGGAGGTGAAAAATTCCTCCTACCCGCGGCAAGACGGAGGGACCCCGTGACCTTTAC
R5 241	TAGTGGAGGTGAAAAATTCCTCCTACCCGCGGCAAGACGGAGGGACCCCGTGACCTTTAC
J99 1428204	TACAACCTAGCACTGCTAATGGGAATATCATGCGCAGGATAGGTGGGAGGCTTTGAAGTA
S 301	TACAACCTAGCACTGCTAATGGGAATATCATGCGCAGGATAGGTGGGAGGCTTTGAAGTA
R1 301	TACAACCTAGCACTGCTAATGGGAATATCATGCGCAGGATAGGTGGGAGGCTTTGAAGTA
R2 301	TACAACCTAGCACTGCTAATGGGAATATCATGCGCAGGATAGGTGGGAGGCTTTGAAGTA
R3 301	TACAACCTAGCACTGCTAATGGGAATATCATGCGCAGGATAGGTGGGAGGCTTTGAAGTA
R4 301	TACAACCTAGCACTGCTAATGGGAATATCATGCGCAGGATAGGTGGGAGGCTTTGAAGTA
R5 301	TACAACCTAGCACTGCTAATGGGAATATCATGCGCAGGATAGGTGGGAGGCTTTGAAGTA
J99 1428144	AGGGCTTTGGCTCTTATGGAG
S 361	AGGGCTTTGGCTCTTATGGAG
R1 361	AGGGCTTTGGCTCTTATGGAG
R2 361	AGGGCTTTGGCTCTTATGGAG
R3 361	AGGGCTTTGGCTCTTATGGAG
R4 361	AGGGCTTTGGCTCTTATGGAG
R5 361	AGGGCTTTGGCTCTTATGGAG

Figure 4 Comparison of the 23S rRNA gene sequence of *Helicobacter pylori* isolates with that of HPJ99 strain. S: A clarithromycin-sensitive isolate; R1-5: clarithromycin-resistant strains.

genotypes (Figure 6).

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). The

Table 2 Clinical characteristics of 26 patients from whom clarithromycin-sensitive *Helicobacter pylori* strains were isolated

No.	Strain	Gender	Age	Ethnicity	Region	Disease type	History of medication	Family history of gastric disease
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	GXHP074	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	GXHP040	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	-	+

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.

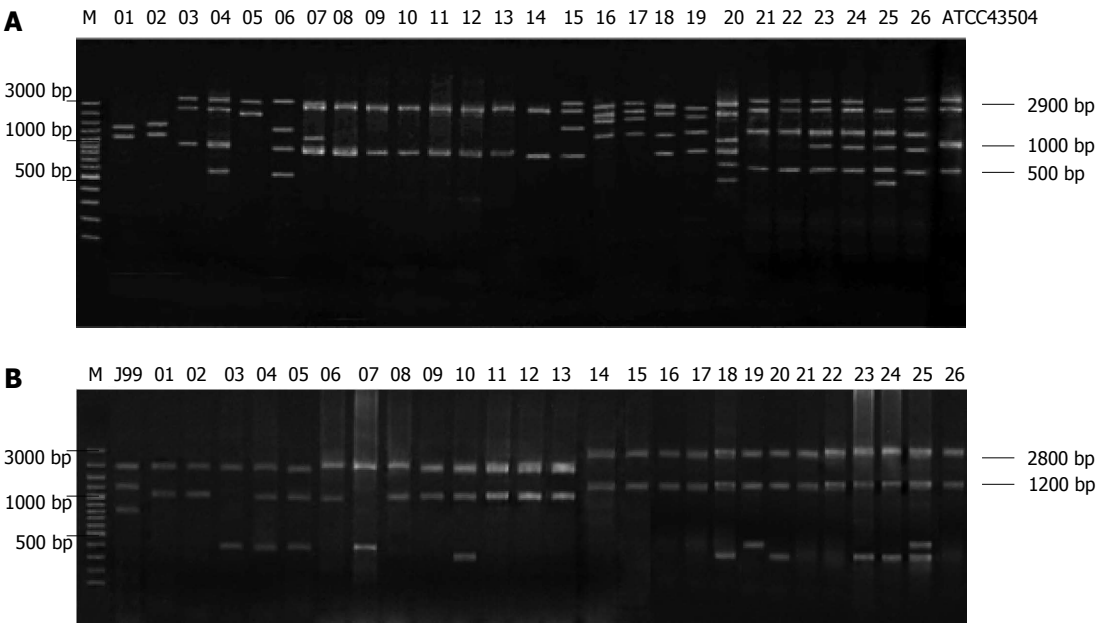


Figure 5 REP-PCR analysis of 26 clarithromycin-resistant (A) and clarithromycin-sensitive (B) *Helicobacter pylori* isolates. M: λ DNA/EcoR I-Hind III marker; 1-26: Clarithromycin-resistant *Helicobacter pylori* isolates; ATCC43504: A standard *Helicobacter pylori* strain.

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)^[20]. Therefore, we recommend that, in order to improve the rate of *H. pylori* eradication, tests for *H. pylori* antibiotic resistance should be

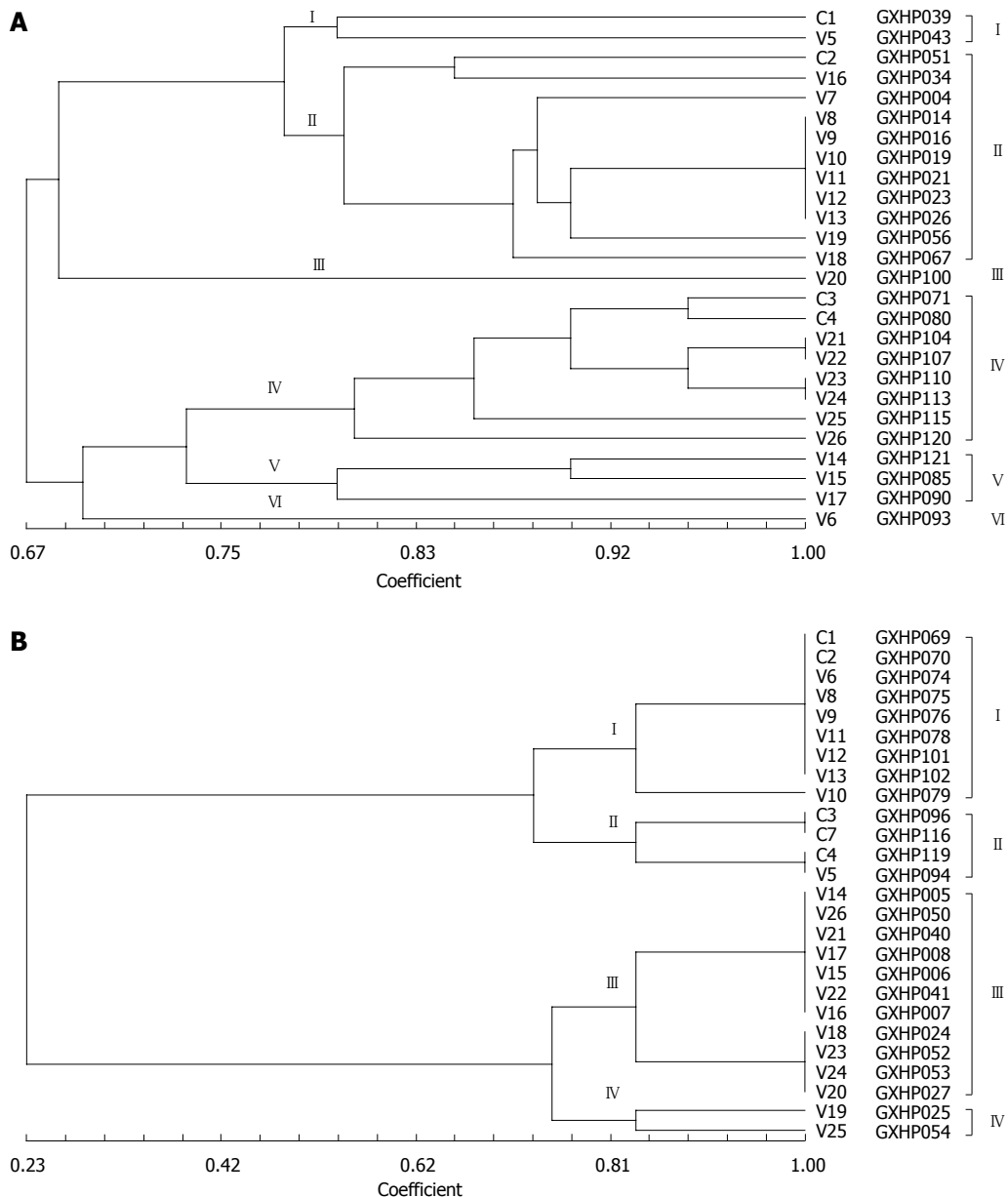


Figure 6 Dendrogram of REP-PCR DNA fingerprints for clarithromycin-resistant *Helicobacter pylori* strains. A: 6 genotypes; B: 4 genotypes.

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	HM	FHGD	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	5	4	1	6	4
Group III	1	0	0	1	1	0	0	0	1	0	1	1	1	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	0	1	0	0	0	1	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; FHGD: Family history of gastric disease.

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

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

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

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 domain V of the 23S ribosomal RNA, which reduce the binding force between clarithromycin and the ribosome. Most of the discovered mutations are A to G transition mutations, and currently known mutations include A2144G, A2143G, A2142G, A2142C, G2115A, G2141A, A2142T and A2143C. Some mutations can occur even in other chromosomal segments. Since the minimum inhibitory concentrations of clarithromycin are higher in *H. pylori* strains bearing A2144G and A2143G mutations, these strains have more stable resistance and higher growth rate^[24-26]. Therefore, A2143G and A2144G mutations are most common in clinically isolated clarithromycin-resistant *H. pylori* strains. However, point mutations in clarithromycin-resistant *H. pylori* strains can vary among different regions and different ethnicity groups.

This study detected the A2143G and A2144G mutations in the 23S rRNA gene in *H. pylori* isolates using PCR-RFLP, which is a simple and high-sensitivity method. Both mutations were detected in all 10 randomly selected clarithromycin-resistant isolates, but not in clarithromycin-sensitive isolates. Direct DNA sequencing of the 23S rRNA gene in 5 randomly selected clarithromycin-resistant isolates confirmed the above finding. Therefore, A2143G and A2144G mutations are closely related to *H. pylori* resistance to clarithromycin in ethnic minority patients in Guangxi. Future studies should develop methods to repair or avoid these mutations to reduce the rate

of clarithromycin resistance and improve the effectiveness of prevention and control.

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 GXHP104 and GXHP107; and between GXHP110 and GXHP113. These three groups shared a similarity of 90%. In addition, GXHP104 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, GXHP016, 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 GXHP026 were also resistant to amoxicillin and streptomycin. GXHP051, GXHP056 and GXHP067 came from 3 Han patients in Baise or Tianlin, all of whom had a history of clarithromycin use and a family history of gastric disease. These three strains were also resistant to amoxicillin, streptomycin and gen-

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. The patient was in the 41-50 age group; (4) group IV: This group includes GXHP071, GXHP080, GXHP104, GXHP107, GXHP110, GXHP113, GXHP115 and GXHP120. The 8 strains came from 8 patients with chronic gastritis (5 males and 3 females) in Baise, Tianlin, Lingyun, or Tianyang. There were 6 Han patients and 2 Zhuang patients. Four patients had a history of clarithromycin use and five patients had a family history of gastric diseases. Four strains were also resistant to amoxicillin and gentamicin. In this group, one patient was in the 30-40 age group, 3 in the 41-50 age group, and 4 in the 51-60 age group; (5) group V: This group includes GXHP085, GXHP090 and GXHP121. All 3 strains came from 3 females with chronic gastritis in Baise or Tianlin. There were 1 Han patient and 2 Zhuang patients. One patient had both a history of clarithromycin use and a family history of gastric diseases. One strain was also resistant to amoxicillin and gentamicin. In this group, 2 patients were in the 41-50 age group, and 1 in the 51-60 age group; and (6) group VI: This group includes only GXHP093. This strain came from a Zhuang female with chronic gastritis in Tianlin. She had an unknown history of clarithromycin use or family history of gastric diseases. The strain was also resistant to amoxicillin and streptomycin. The patient was in the 41-50 age group.

Based on the above data, it can be found that factors influencing genotype distribution include disease type, ethnicity, gender, age, region, history of medication, family history of gastric disease, and multidrug resistance. The frequencies of the presence of these factors influencing genotype distribution of clarithromycin-resistant strains are shown in Table 3. Main factors influencing genotype distribution of clarithromycin-resistant strains may be: (1) disease type: Gastritis and peptic ulcer were main influencing factors, because group II and group IV strains were isolated from patients with gastritis and those with peptic ulcer, respectively; (2) ethnicity: Zhuang and Han were main influencing factors, because GXHP014, GXHP016, GXHP019, GXHP021, GXHP023 and GXHP026, which shared a similarity of 100%, were all isolated from Zhuang patients; GXHP104 and GXHP107, which shared a similarity of 100%, as well as GXHP110 and GXHP113, which also shared a similarity of 100%, were all isolated from Han patients; (3) family history of gastric disease: Since GXHP104 and GXHP107, which shared a similarity of 100%, were isolated from Han patients with a family history of gastric diseases, while GXHP110 and GXHP113, which also shared a similarity of 100%, were isolated from Han patients without a family history of gastric diseases, we speculate that family history of gastric disease may be

associated with the genotype distribution; (4) multidrug resistance: Since the frequency of the presence of multidrug resistance is high (50%-100%) in *H. pylori* isolates of various genotypes, there may exist an association between multidrug resistance and the genotype distribution.

We also analyzed the genotypes of clarithromycin-sensitive *H. pylori* strains using the same method and found that the main factors influencing the genotype distribution of clarithromycin-sensitive strains were disease type and patient gender (Figures 5B and 6B, Table 2). Compared with clarithromycin-resistant strains, the genotype distribution of clarithromycin-sensitive strains was influenced by fewer factors. This discrepancy may be partly explained by the genetic mutations in clarithromycin-resistant strains. In addition, we found that region, age and history of medication had relatively small impact on genotype distribution. However, since the number of strains analyzed in the present study is relatively small, we could not use statistical methods to analyze the influence of various factors on genotype distribution. Future studies should carefully address this issue.

COMMENTS

Background

Helicobacter pylori (*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. The wide application of antibiotics in *H. pylori* eradication therapy has led to the increasing prevalence of *H. pylori* resistance to antibiotics. Antibiotic resistance is a major cause of treatment failure.

Research frontiers

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.

Applications

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. Clarithromycin-resistant *H. pylori* isolates can be divided into six groups based on REP-PCR DNA fingerprints. Several factors such as disease type may influence the genotype distribution of *H. pylori* isolates.

Peer review

The manuscript is very interesting. The authors try to investigate the rate of *H. pylori* resistance to clarithromycin among ethnic minority patients in Guangxi, the home to many ethnic minorities besides the Han Chinese. The research is well designed. The data are very interesting.

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