Original Article

Correlation analysis of gastric mucosal lesions with *Helicobacter pylori* infection and its virulence genotype in Guiyang, Guizhou province, China

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Background: *Helicobacter Pylori (H. pylori)* infection is the most important factor affecting clinical outcome in patients with gastric mucosal lesions. This study aimed to investigate *H. pylori* infection in patients with gastric mucosal lesions and their virulence genotype in Guiyang, China.

Methods: Pathological examinations of 1,364 biopsies from patients with upper gastrointestinal symptoms and *H. pylori* infection were analyzed according to different pathological types. The bacterial genome DNA was extracted from *H. pylori* strains isolated from gastric biopsies, and the *cagA*, *vacA*, and *iceA* virulence genes were detected and typed to analyze the correlation of their genotypes between different pathological lesions.

Results: The positive rate of *H. pylori* infection was approximately 19.9% (272/1,364), as determined by histopathological examination (HPE). It was more frequently detected in men than in women. A total of 85 *H. pylori* isolates were obtained from 280 clinical samples (positive rate 30.4%, 85/280). Of these 85 strains, *cagA*, *vacA*, and *iceA* genes were identified in 85.9%, 100%, and 83.5% of samples, respectively. Approximately 74.1% of strains were *cagA* East Asian type (*cagA*-ABD), and 11.8% of were *cagA* Western strains (*cagA*-AB, *cagA*-ABC), only present in patients with chronic non-atrophic gastritis. Gastric intraepithelial neoplasia and gastric cancer harbored both Asian strains. A total of 7 combinations of *vacA* genotypes were noted, among which *s1c/m1b* (30.6%) and *s1c/m2* (41.2%) were the dominant genotypes. The predominant *iceA* genotype was *iceA1* (64.7%).

Conclusions: We observed that the positive rate of *H. pylori* infection was related to the pathological type of patients' gastric mucosal lesions. Isolated *H. pylori* strains showed a unique genotype, mainly East Asian type *cagA* (ABD), *vacA s1c/m2* genotype, and *iceA1*. These results provide an important reference for further studies of *H. pylori* in Guizhou province, China.

Keywords: *H. pylori* infections; pathological type; gastric mucosal lesion; Guizhou province

Submitted Oct 21, 2022. Accepted for publication Dec 02, 2022.

doi: 10.21037/atm-22-5553

View this article at: https://dx.doi.org/10.21037/atm-22-5553

Introduction

In 1982, Warren and Marshall discovered a bacterium that colonized the surface of the human gastric mucosa which was later named *Helicobacter pylori (H. pylori)*. *H. pylori* is a gram-negative, micro-aerobic, spiral-curved bacteria that colonizes the surface of the human gastric mucosa (1). The bacterium decomposes urea to resist gastric acid and secrete toxins into the digestive tract. These toxins destroy the defense mechanism and immunity of the host, and inflammation of the body is then induced (2). Potential consequences of *H. pylori* infection include upper gastrointestinal diseases, such as gastritis, gastroduodenal ulcer, gastric mucosa-associated lymphoid tissue lymphoma, and gastric cancer (3). Over the last few decades, the prevalence of *H. pylori* infection has been decreasing worldwide (4), and the prevalence significantly decreased from 58.3% in the period 1983–1994 to 40.0% in the period 2015–2019 in China (5). A study found the positive rates of *H. pylori* by rapid urease test (RUT), histopathological examination (HPE), polymerase chain reaction (PCR), and culture to be 47.1%, 51.3%, 50.3%, and 32.4%, respectively (6). Each method has its unique advantages and disadvantages, and the occurrences of false negatives and positives cannot be avoided (7,8). Bacterial culture of endoscopic biopsy specimens is the gold standard for diagnosing *H. pylori* colonization (9), but the frequency of positive culture is low. Typically, the bacteria are easily observed in well-differentiated hematoxylin-eosin (H&E) stained sections, and special staining for routine diagnosis is not necessary (10).

Highlight box

Key findings

- We found that the prevalence of *H. pylori* infection was related to the pathological type of gastric mucosal lesion.

What is known and what is new?

- *H. pylori* was more frequently detected in men than in women. Most of the current studies have reported the relationship between clinical diagnosis and *H. pylori* infection and virulence genes.
- Using the gold standard (histopathological examination) for diagnosing *H. pylori* infection, we studied the relationship between gastric mucosa pathology and *H. pylori* infection and its virulence genes.

What is the implication, and what should change now?

- Eradication of *H. pylori* is essential to prevent further lesions in the gastric mucosa, especially for cagA-positive strains.

The difference in clinical outcomes after *H. pylori* infection is related to the host's susceptibility, the virulence of the bacteria strain, environmental co-factors, and socio-economic conditions (11). Among them, the most important factor affecting clinical outcomes is the *H. pylori* virulence genes, and the 3 best-understood genes in terms of structure and function are cytotoxin-associated gene A (cagA), vacuolating cytotoxin A (*vacA*), and induced by contact with epithelium gene A (*iceA*). The virulence genotype expression of *H. pylori* in different digestive tract diseases varies significantly (12).

The *cagA* gene is an important virulence factor and *cagA* positive strains can increase the risk of gastric ulcers and cancers (13). The distribution of *H. pylori cagA* virulence genes shows obvious regional differences. The *cagA* gene has been detected in 50–60% of the Western *H. pylori* strains (14); however, over 90% of the strains isolated in an East Asian population possessed this gene (15), which is directly translocated with the epithelial cell via a type IV secretion system (16). The previous study has shown the existence of four EPIYA motifs (A, B, C, D). The East Asian type was replaced by the EPIYA-D segment instead of the EPIYA-C fragment, and it was more virulent than the Western type (17). A relevant study has shown that the population of the Guizhou province of China is dominated by the *cagA* East Asian type, with a positive rate of over 80% (18).

Almost all *H. pylori* strains produce and secrete *vacA*, which induces and produces a variety of cell activities (19) that damage the gastric mucosa. Several studies have shown that the *vacA* virulence gene is the most abundant of the *H. pylori* gene polymorphisms (20). Its genetic polymorphism is expressed in the signal (*s*) region, the middle (*m*) region, and the intermediate (*i*) region (21). In general, *s1m1* and *s1m2* strains produce high and moderate levels of toxins, respectively, whereas *s2m2* strains produce little or no toxin (22).

The *iceA* gene is another important virulence factor. The 2 main allelic variants of the gene are *iceA1* and *iceA2* (23). Of them, *iceA1* is upregulated upon contact of *H. pylori* with the gastric epithelium and has been considered a marker for peptic ulcer disease and gastritis (24).

This study aimed to investigate the association between gastric mucosal lesions with *H. pylori* infection and their *cagA*, *vacA*, and *iceA* genotypes in Guiyang, Guizhou. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5553/rc).

Methods

Our study enrolled 1,364 patients with upper gastrointestinal discomfort (e.g., abdominal pain, postprandial fullness, acid reflux, and burning) and without obvious contraindications for upper gastrointestinal endoscopy who underwent electronic gastroscopy and gastroscopic gastric mucosal biopsy from the Affiliated Cancer Hospital of Guizhou Medical University and the Guiyang Hospital of Guizhou Aviation Industry between August 2018 and February 2019. The results of these procedures were reviewed retrospectively. All patients agreed to undergo electronic gastroscopy and gastroscopic gastric mucosal biopsy. The biopsies were obtained for histopathological evaluation, *H. pylori* culture, and virulence genotype detection.

HPE

Between 1 and 3 biopsy specimens from the antral and/or stomach body of the gastric mucosa were obtained via routine gastroscopy for histological examinations. The sections were fixed with 10% neutral formalin, then paraffin-embedded and stained with H&E. *H. pylori* infection and pathological lesions of the gastric mucosa were diagnosed by 2 independent pathologists using an ordinary light microscope.

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of The Affiliated Hospital of Guizhou Medical University (No. 2018-100) and informed consent was taken from all the patients. Guiyang Hospital of Guizhou Aviation Industry was informed and agreed the study. Control strain: *H. pylori* standard strain NCTC11637 was donated by the Chinese Center for Disease Control and Prevention.

H. pylori isolation and bacterial DNA extraction

For *H. pylori* isolation, gastric mucosa biopsy samples were cut into tiny sections, homogenized, and smeared on the surface of brain heart infusion agar with 10% sheep blood (Qingdao Hope Biol Technology Co., Ltd., Qingdao, China) and antibiotic supplement (*H. pylori* selective supplement, Thermo Fisher Scientific Oxoid, Ltd., Basingstoke, UK), which were incubated afterward in a microaerobic incubator for 3–5 days. Suspected colonies of *H. pylori* were identified by Gram stain, spiral morphology, urease, oxidase, and catalase tests, and *H. pylori* specific 16S rRNA gene fragment polymerase chain reaction (PCR) amplification. The colony of *H. pylori* was translucent and smooth, and the morphology was Gram-negative and spiral-shaped bacilli. The primers (Sangon Biotech Co., Ltd., Shanghai, China) used for amplification are shown in *Table 1*. The DNA of *H. pylori* isolates was extracted using an Ezup column bacteria genomic DNA purification kit (Sangon Biotech Co., Ltd., Shanghai, China), according to the manufacturer's protocol. The *H. pylori* strain NCTC11637 was used as a positive control.

PCR amplification

Following DNA extraction from a pure culture of *H. pylori* isolates, PCR assays were performed in a volume of 26 μL containing 1 μL reverse primer, 1 μL forward primer, 1 μL genomic DNA, 13 μL 2X Taq PCR Master Mix (Beijing Solarbio Science and Technology Co., Ltd., Beijing, China), and 10 μL ddH_2O. All runs included 1 negative (ddH_2O) and 1 positive (NCTC11637) DNA control and DNA ladder markers (Tiangen Biotech Co., Ltd., Beijing, China). A total of 5 μL of amplified PCR products was resolved by electrophoresis on 1% agarose gels run in acetate ethylene-diamine-tetraacetic acid (EDTA) buffer and stained with cyber green. The PCR product was visualized under gel electrophoresis.

Sequencing of cagA, vacA, and iceA

Primers (Sangon Biotech Co., Ltd., Shanghai, China) were to amplify *cagA*, *vacA*, and *iceA* genes, and the cycling conditions in the present study are shown in *Table 1*. The amplification of the *iceA* and the allelic combinations (*s1*, *s2*, *m1*, and *m2*) of *vacA* were visualized using agarose gel electrophoresis. PCR assays to amplify *cagA* and sequence its C-terminal region were performed according to the report by Sicinschi *et al.* (25). The *cagA* C-terminal PCR products were sent to Sangon Biotech Co., Ltd. for Sanger sequencing. Biological software DNAMAN (Lynnon Biosoft, San Ramon, CA, USA) was used to convert the gene sequence into an amino acid sequence for EPIYA typing.

Table 1 Primer sequences and PCR conditions

Gene	Primer	Primer sequence	Size (bp)	Amplification condition
16S rRNA	*16S rRNA-F*	5'-CTTGCTAGAGTGCTGATTA-3'	550	35 cycles: 94 ℃ for 30 sec; 55 ℃ for 30 sec; 72 ℃ for 30 sec
	16S rRNA-R	5'-TCCCACACTCTAGAATAGT -3'		
cagA 5'-	*cagA F*	5'-GATAACAGGCAAGCTTTTGAGG-3'	349	30 cycles: 94 ℃ for 1 min; 55 ℃ for 1 min; 72 ℃ for 1 min
	cagA R	5'-CTGCAAAAGATTGTTTGGCAGA-3'		
cagA 3'-	*cagA –VF*	5'-ACCCTAGTCGGTAATGGGTTA-3'	591–856	30 cycles: 94 ℃ for 1 min; 50 ℃ for 1 min; 72 ℃ for 1 min
	cagA -VR	5'-GTAATTGTCTAGTTTCGC-3'		
vacA-s1a	*vacA -s1a-F*	5'-CTCTCGCTTTAGTAGGAGC-3'	213	30 cycles: 94 ℃ for 30 sec; 60 ℃ for 30 sec; 72 ℃ for 45 sec
	vacA -s1a-R	5'-CTGCTTGAATGCGCCAAAC-3'		
vacA-s1b	*vacA -s1b-F*	5'-AGCGCCATACCGCAAGAG-3'	187	
	vacA -s1b-R	5'CTGCTTGAATGCGCCAAAC3'		
vacA-s1c	*vacA -s1c-F*	5'-CTCTCGCTTTAGTGGGGYT-3'	213	
	vacA-s1c-R	5'-CTGCTTGAATGCGCCAAAC-3'		
vacA-s2	*vacA-s2-F*	5'-GCTAACACGCCAAATGATCC-3'	199	
	vacA-s2-R	5'-CTGCTTGAATGCGCCAAAC-3'		
vacA-m1a	*vacA-m1a-F*	5'-GGTCAAAATGCGGTCATGG-3'	290	
	vacA-m1a-R	5'-CCATTGGTACCTGTAGAAAC-3'		
vacA-m1b	*vacA-m1b-F*	5'-GGCCCCAATGCAGTCATGGAT-3'	291	
	vacA-m1b-R	5'-GCTGTTAGTGCCTAAAGAAGCAT-3'		
vacA-m2	*vacA-m2-F*	5'-GGAGCCCCAGGAAACATTG-3'	352	
	vacA-m2-R	5'CATAACTAGCGCCTTGCAC3'		
iceA1	*iceA1-F*	5'GCTTGTAACGATAAGAAACGCCAGAT3'	297	35 cycles: 94 ℃ for 30 sec; 55 ℃ for 30 sec; 72 ℃ for 30 sec
	iceA1-R	5'GGAATGAGCTTGTATTTAGAGCCGAT3'		
iceA2	*iceA2-F*	5'GTTGGGTATATCACAATTTAT3'	229/334	30 cycles: 94 ℃ for 30 sec; 52 ℃ for 30 sec; 72 ℃ for 45 sec
	iceA2-R	5'TTRCCCTATTTTCTAGTAGGT3'		

PCR, polymerase chain reaction.

Statistical analysis

All data were analyzed using SPSS 22.0 (IBM Corp., Armonk, NY, USA). The rate of *H. pylori* infection was expressed as a percentage, and the chi-squared test was used to assess differences in rates between the groups. Patients' age, classified according to histopathological type, was expressed as means ± standard deviations. The chi-square test and Fisher's exact test were used to analyze correlation of *cagA*, *vacA*, and *iceA* genotypes with different histopathological lesions and age groups. P values <0.05 were considered statistically significant.

Results

A total of 1,364 patients were included in the analysis.

H. pylori infection in patients according to sex

The positive rate of *H. pylori* infection in our study population was 19.9% (*Table 2*). The positive infection

Table 2 *H. pylori* infection in patients of different sex diagnosed by histopathology

Gender	*H. pylori* infection		Total (n)	Positive rate (%)	χ^2	P value
	Positive (n)	Negative (n)				
Men	148	520	668	22.2	4.021	0.045*
Women	124	572	696	17.8		
Total	272	1,092	1,364	19.9		

n: number of strains; *, P<0.05. *H. pylori, Helicobacter pylori.*

rate was 22.2% in men and 17.8% in women, which was a significant difference (P=0.045).

H. pylori infection in four histopathological groups

The histopathological diagnoses determined by the examination of H&E-stained biopsy sections included chronic non-atrophic gastritis, chronic atrophic gastritis, gastric ulcer, gastric polyps, chronic metaplastic atrophic gastritis, intraepithelial neoplasia, and gastric cancer (*Figure 1*). The patients were divided into 4 groups according to their diagnosis: chronic non-atrophic gastritis (n=782), precancerous conditions (n=400), gastric precancerous lesions (n=151), and gastric cancer (n=31). The precancerous conditions group comprised patients with chronic atrophic gastritis, gastric ulcer, gastric polyps, and gastric stumps. The gastric precancerous lesions group comprised patients with chronic metaplastic atrophic gastritis, and intraepithelial neoplasia. The positive infection rate was 15.7% in the chronic non-atrophic gastritis group, 23.0% in the precancerous condition group, 37.1% in the gastric precancerous lesion group, and 3.2% (1/31) in the gastric cancer group. *H. pylori* infection rates differed among the 4 groups (P<0.01) (*Table 3*).

Histopathology according to age

The mean age was 52.2±12.3 years in the chronic non-atrophic gastritis group, 55.3±13.0 years in the precancerous condition group, 56.3±11.9 years in the gastric precancerous lesion group, and 60.0±14.0 years in the gastric cancer group. The average age in the chronic non-atrophic gastritis group significantly differed from that in the other histopathological groups (*Table 4*).

H. pylori infection according to age groups

The positive rates of *H. pylori* infection were 20.7%, 20.4%, and 18.9% in patients 15–39, 40–59, and 60–91 years old, respectively; 14 patients were >80 years old. There were no significant differences in infection rates among the age groups (P>0.05) (*Table 5*).

Numbers of patients in different histopathological groups and age groups

The numbers of affected patients differed among histopathological and age groups. The number of infected patients in an age group decreased increasing histopathological severity. Among the chronic non-atrophic gastritis group, precancerous condition group, and gastric precancerous lesion group, patients aged 40–59 years were the most prevalently infected, whereas gastric cancer was more common in patients aged 60–91 years (*Table 6*).

Patients' clinical information and histopathological diagnosis

During the study period, 85 *H. pylori* isolates were obtained from 280 cases of gastric biopsies (positive rate 30.4%, 85/280). Of these 85 isolates, 49 were from men and 36 were from women. The HPE of these 85 patients showed 59 cases of chronic non-atrophic gastritis, 2 cases of chronic atrophic gastritis, 16 cases of chronic atrophic gastritis with intestinal metaplasia, 2 cases of gastric intraepithelial neoplasia, 1 case of gastric polyps, and 5 cases of gastric cancer (*Table 7*). There was no significant difference between different histopathological type in terms of sex and age. Patients with complete information were used for genotyping.

A total of 85 strains were isolated, which could be stably subcultured and survive. All *H. pylori* isolates were gram-negative campylobacter, which are urease, oxidase, and catalase positive, and the 16S rRNA fragment specificity of their strains was positive. The presence of the cagA, vacA, and iceA genes was examined in all 85 *H. pylori*-infected

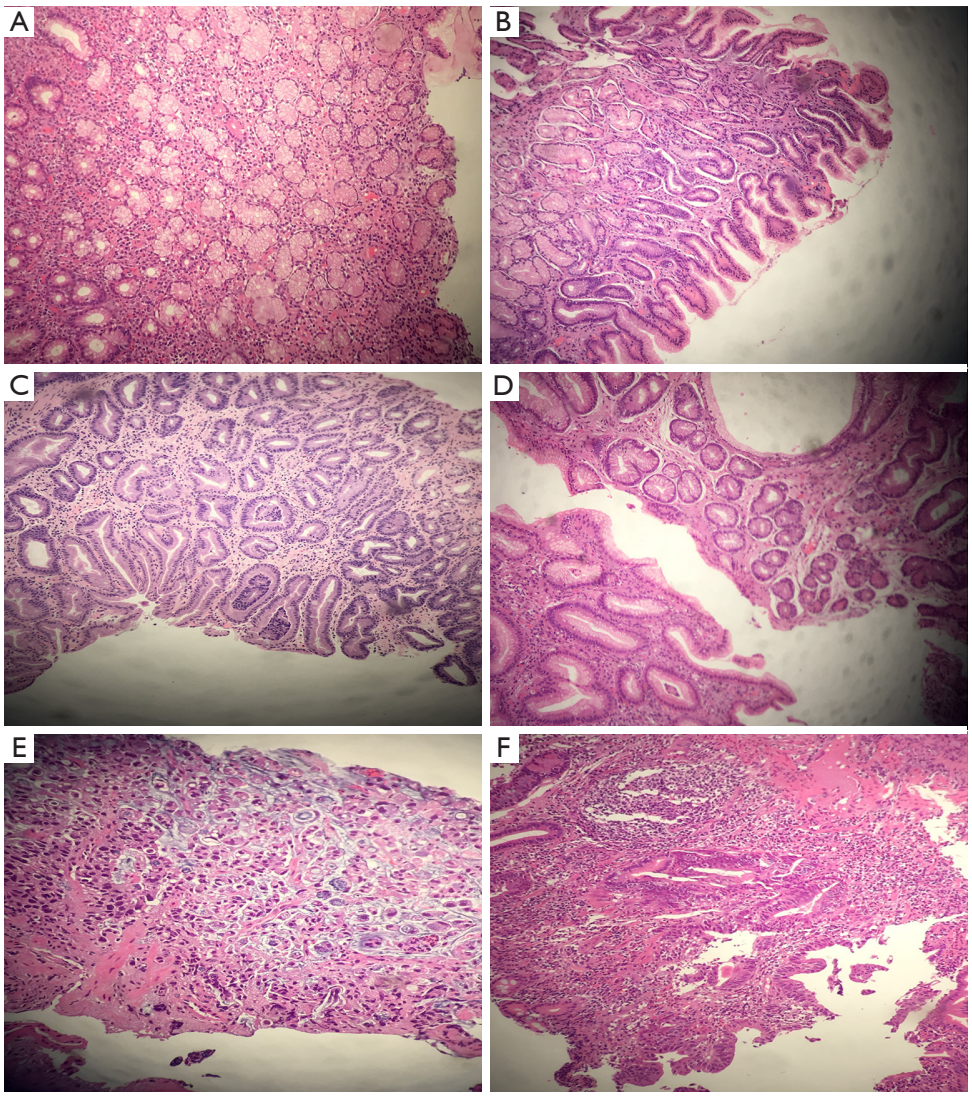

Figure 1 Endoscopic gastric biopsy stained with H&E. (A) Normal gastric mucosa with mild chronic inflammatory cells. (B) Atrophy of glandular mucosa with chronic infiltrate cells. (C) Metaplastic atrophic gastritis with chronic inflammatory cells infiltrates. (D) Mucosal ulceration with chronic and acute inflammatory cells infiltrate. (E) Gastric mucosa with adenocarcinoma infiltrate. (F) Gastric mucosa with intramucosal cancer. H&E, hematoxylin and eosin. Magnification times ×100.

Table 3 *H. pylori* infection rate in the four histopathology groups

Histopathological diagnosis	*H. pylori* infection		Total	Positive rate (%)	χ^2	P value
	Positive (n=272)	Negative (n=1,092)				
Chronic non-atrophic gastritis group	123	659	782	15.7	44.263	0.000*
Precancerous condition group	92	308	400	23.0		
Gastric precancerous lesion group	56	95	151	37.1		
Gastric cancer group	1	30	31	3.2		
Total	272	1,092	1,364	19.9		

n: number of strains; *, P<0.05. *H. pylori, Helicobacter pylori.*

Table 4 Different histopathological groups and their ages

Age	Chronic non-atrophic gastritis group (n=782)	Precancerous condition group (n=400)	Gastric precancerous lesion group (n=151)	Gastric cancer group (n=31)
Average age	52.2±12.3	55.3±13.0[†]	56.3±11.9[†‡]	60.0±14.0[†‡§]

n: number of strains. Age is expressed as the mean ± standard deviation. [†], compared with Chronic non-atrophic gastritis group: P<0.01. [‡], compared with Precancerous condition group: P>0.05. [§], compared with Gastric precancerous lesion group: P>0.05.

Table 5 *H. pylori* infection in patients of different ages

Age, years	*H. pylori* infection		Total	Positive rate (%)	χ^2	P value
	Positive (n=272)	Negative (n=1,092)				
15–39	35	134	169	20.7	0.464	0.793
40–59	149	581	730	20.4		
60–91	88	377	465	18.9		
Total	272	1,092	1,364	19.9		

n: number of strains. *H. pylori, Helicobacter pylori.*

Table 6 The number of patients in different histopathological groups and different age groups

Histopathological diagnosis	15–39 years old (n)	40–59 years old (n)	60–91 years old (n)	Total (n)	χ^2	P value
Chronic non-atrophic gastritis group	88	405	289	782	19.645	0.003*
Precancerous condition group	50	234	116	400		
Gastric precancerous lesion group	27	81	43	151		
Gastric cancer group	4	10	17	31		
Total	169	730	465	1,364		

n: number of strains; *, P<0.05.

patients with upper gastrointestinal symptoms (*Figure 2*).

Detection of H. pylori cagA, vacA, and iceA

The *cagA* genotype distributions of the 85 clinical strains were *cagA*-AB (8.2%), ABC (3.5%), ABD (74.1%), and a negative status for the others (14.1%). None of the clinical isolates showed a *cagA*-BD genotype of the East Asian type. Western strains (*cagA*-AB genotype) only appeared in patients with chronic non-atrophic gastritis. The gastric intraepithelial neoplasia and gastric cancer groups harbored both Asian strains (*cagA*-ABD genotype). The distribution of *H. pylori cagA* genotype in each histopathological type is shown in *Table* 7.

The *vacA* genotype was detected among all clinical isolates of *H. pylori*, and its distribution in histopathological types is shown in *Table* 7. All the strains were positive for *vacA*; *vacA s1c/m2* and *s1c/m1b* were the main genotypes, accounting for 41.2% and 30.6%, respectively. The *vacA s1c/m2* and *s1c/m1b* were the most frequent combination in chronic non-atrophic gastritis. The PCR analysis data presented a summary of the diversity and allelic combinations of *H. pylori* in different pathology.

The *iceA* gene was detected in 83.5% of *H. pylori*; *iceA1* and *iceA2* were detected in 64.7% and 9.4%, respectively. *iceA1* was most commonly found in chronic non-atrophic gastritis. The genotype of gastric cancer was *iceA1*.

There was no significant difference between the histopathological type and virulence genotypes.

Discussion

H. pylori has been confirmed as an important pathogen in the human gastrointestinal tract. Different isolates cause

Table 7 Distribution of various combinations of virulence genotypes among 85 *H. pylori* isolates and their association with clinical information and histopathological outcomes

Variable	n	Gastric polyp	Chronic non-atrophic gastritis	Chronic atrophic gastritis	Chronic atrophic gastritis with intestinal metaplasia	Gastric intraepithelial neoplasia	Gastric cancer	P value
Men	49	0 (0.0%)	38 (64.4%)	0 (0.0%)	8 (50.0%)	1 (50.0%)	2 (40.0%)	0.233
Women	36	1 (100%)	21 (35.6%)	2 (100%)	8 (50.0%)	1 (50.0%)	3 (60.0%)	
Age (years)	85	71	48.86±12.24	49.00±8.49	53.44±12.68	66.50±14.85	60.00±9.25	
cagA	85	1 (1.2%)	59 (69.4%)	2 (2.4%)	16 (18.8%)	2 (2.4%)	5 (5.9%)	0.491
Negative	12	1 (1.2%)	7 (8.2%)	1 (1.2%)	3 (3.5%)	0 (0.0%)	0 (0.0%)	
cagA-AB	7	0 (0.0%)	7 (8.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
cagA-ABC	3	0 (0.0%)	3 (3.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
cagA-ABD	63	0 (0.0%)	42 (49.4%)	1 (1.2%)	13 (15.3%)	2 (2.4%)	5 (5.9%)	
vacA	85	1 (1.2%)	59 (69.4%)	2 (2.4%)	16 (18.8%)	2 (2.4%)	5 (5.9%)	0.603
s1a/m1b	4	0 (0.0%)	4 (4.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
s1a/m1b/m2	1	0 (0.0%)	1 (1.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
s1a/m2	9	0 (0.0%)	8 (9.4%)	0 (0.0%)	1 (1.2%)	0 (0.0%)	0 (0.0%)	
s1c/m1b/m2	8	1 (1.2%)	4 (4.7%)	0 (0.0%)	3 (3.5%)	0 (0.0%)	0 (0.0%)	
s1c/m2	35	0 (0.0%)	27 (31.8%)	0 (0.0%)	5 (5.9%)	1 (1.2%)	2 (2.4%)	
s1c/m1b	26	0 (0.0%)	14 (16.5%)	2 (2.4%)	6 (7.1%)	1 (1.2%)	3 (3.5%)	
m1b	2	0 (0.0%)	1 (1.2%)	0 (0.0%)	1 (1.2%)	0 (0.0%)	0 (0.0%)	
iceA	85	1 (1.2%)	59 (69.4)	2 (2.4%)	16 (18.8%)	2 (2.4%)	5 (5.9%)	0.392
Negative	14	0 (0.0%)	9 (10.6%)	0 (0.0%)	5 (5.9%)	0 (0.0%)	0 (0.0%)	
iceA1	55	0 (0.0%)	39 (45.9%)	1 (1.2%)	8 (9.4%)	2 (2.4%)	5 (5.9%)	
iceA2	8	1 (1.2%)	5 (5.9%)	0 (0.0%)	2 (2.4%)	0 (0.0%)	0 (0.0%)	
Mixed	8	0 (0.0%)	6 (7.1%)	1 (1.2%)	1 (1.2%)	0 (0.0%)	0 (0.0%)	

Age is expressed as the mean ± standard deviation. n, number of strains; *H. pylori, Helicobacter pylori.*

various gastrointestinal disorders resulting in different gastric mucosal lesions, such as injury of gastric mucosa, transformation of tissue stratum, chronic inflammation, chronic gastritis, and gastric cancer (26). However, not all patients experience these complications, and more than 50% do not show any symptoms (27). Genetic pathogenesis of different isolates and environmental characteristics are essential factors responsible for this discrepancy. We assessed *H. pylori* infection in patients with upper gastrointestinal diseases in Guiyang, Guizhou, China, and determined whether the pathology of the gastric mucosa was related to the infection. *H. pylori* was analyzed for the presence of *cagA*, *vacA*, and *iceA* genes.

The positive rate of *H. pylori* infection in our study population was 19.9%; this was significantly lower than the prevalence reported in previous studies in China. Xu *et al.* collected 262 gastric mucosa specimens in Beijing, and the positive rate of *H. pylori* detected by H&E staining was 43.9% (28). Xu *et al.* collected 214 gastric mucosa specimens in Ningxia, and the positive rate of *H. pylori* detected by H&E staining was 27.6% (29). Mai *et al.* collected 260 gastric mucosa specimens in Xishuangbanna, and the positive rate of *H. pylori* detected by H&E staining was 46.2% (30). There are several potential reasons for this difference. First, the detection of *H. pylori* is challenging when it is distributed unevenly in the stomach and when gastric or intestinal metaplasia is present. Intestinal metaplasia lowers the sensitivity of histopathological

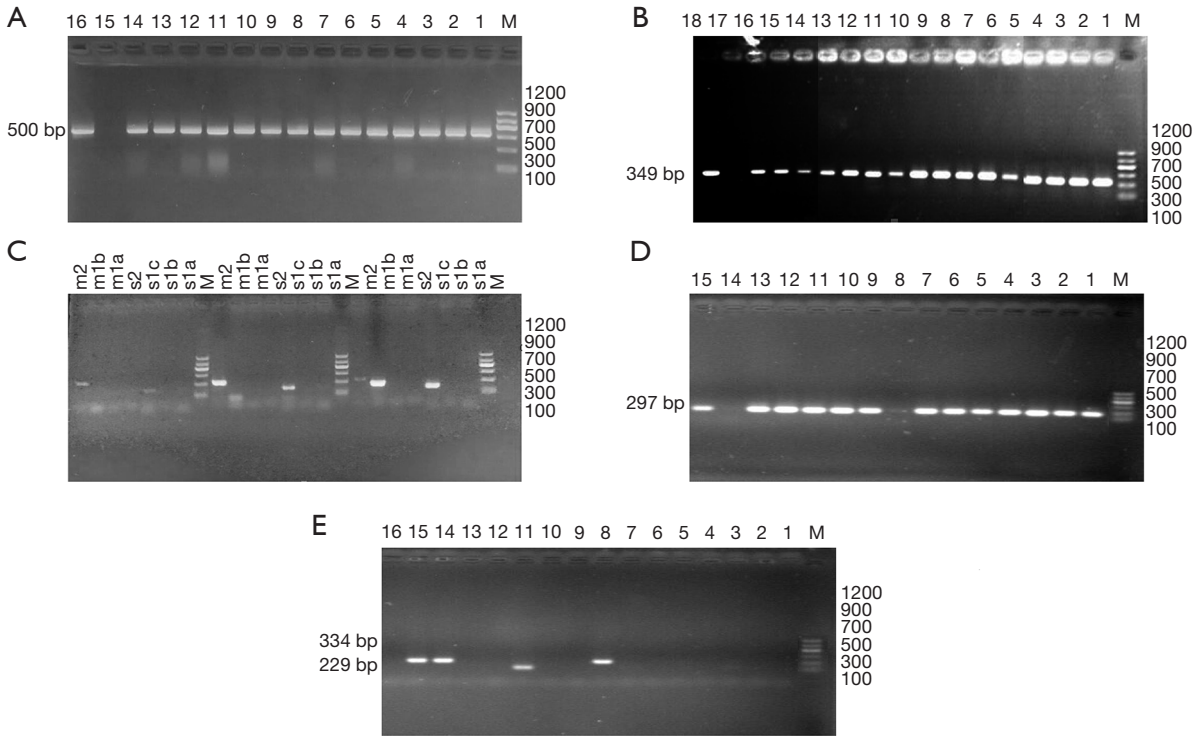

Figure 2 PCR amplification of *H. pylori cagA*, *vacA*, and *iceA*. (A) PCR products amplified from the *H. pylori* specific *16S rRNA* gene. M: Marker II; lane 1–14: clinical strains; lane 15: negative control; lane 16: positive control (NCTC11637). (B) Gel electrophoresis of clinical strains with different *cagA* N terminals. M: Marker II; lane 1–16: *cagA* N-end gene positive; lane 17: negative control; lane 18: positive control (NCTC11637). (C) Gel electrophoresis of *H. pylori* genotyping of *vacA* alleles. M: Marker II. (D) Gel electrophoresis of *H. pylori* genotyping of *iceA1* alleles. M: Marker II; lane 1–7 and 9–13: positive clinical strains; lane 8: negative clinical strains; lane 14: negative control; lane 15: positive control (NCTC11637). (E) Gel electrophoresis of *H. pylori* genotyping of *iceA2* alleles. M: Marker II; lane 8, 11, and 14: positive clinical strains; lane 1–7,9–10, and 13: negative clinical strains; lane 16: negative control; lane 15: positive control (NCTC11637). PCR, polymerase chain reaction.

detection (31), as does gastric atrophy, and produces false-negative results in antral specimens (32). Second, changes in the shape of *H. pylori* from a "comma" or "S" to a sphere can hinder its identification. Such changes occur under some conditions in patients who have recently received proton pump inhibitors or antibiotics (33). Third, histological diagnosis of *H. pylori* infection largely depends on the expertise of the pathologist and the time spent on the diagnosis. Fourth, overgrowth of urease-producing bacteria can cause a false-positive result in a urea breath test, which is a quick diagnostic method routinely used in the clinic. Most urease-producing bacteria reside in the intestines or originate in the oral cavity; these include *α-Streptococcus*, *γ-Streptococcus*, *Hemophilus*, *Enterococcus*, *Neisseria*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*, *Bacillus spp.*, *Bacteroides ovatus*, and *Corynebacterium. spp* (34). Finally, *H. pylori* infection rates have probably declined because of improved living and sanitation conditions and the spread of knowledge about related diseases (35,36). Recent meta-analyses have shown that the eradication of *H. pylori* reduces the incidence of gastric cancer by 33–47% (37).

In our study, *H. pylori* was more frequently detected in men than in women. This result is consistent with that of Zamani *et al.* (38). The difference in detection rate between the sexes may reflect more frequent alcohol and tobacco use by men than by women (38,39). Hence, healthy living habits may play an important role in reducing the frequency of *H. pylori* infections.

According to previous study, the positive rate of *H. pylori* infection and incidence of gastric cancer is highest among patients 40–60 years old (40). Therefore, this study assessed the positive rate of infection in 3 age

groups (15–39, 40–59, and >60 years old). We found no association between age and the positive rate of *H. pylori* infection. This result is consistent with that of Binh *et al.* (41). The *H. pylori* load in the gastric mucosa may be lower in older people, particularly those aged >60 years, because of the physiological atrophy of the stomach glands or the occurrence of intestinal metaplasia. Further investigation is needed to confirm these findings.

In this study, the participants were divided into 4 groups based on the histopathology of the gastric mucosal lesion. The gastric precancerous lesion group had the highest prevalence of *H. pylori* infection, whereas the gastric cancer group had the lowest. The low prevalence of infection in patients with gastric cancer is inconsistent with previous findings (42). This may be explained by reduced gastric acid secretion due to gastric gland atrophy and loss of parietal cells after the gastric lesion has progressed to gastric cancer (43). Moreover, cancer-related changes in stomach acidity may have inhibited or obscured the growth of *H. pylori* or facilitated the growth of other bacteria (44). It is also possible that malignant mucosa is not suitable for *H. pylori* colonization. Finally, our patients might have previously received anti-*H. pylori* treatment. We found that the *H. pylori* load in the non-atrophic gastritis group, precancerous condition group, and precancerous lesion group gradually increased in a severity-dependent manner. This finding agrees with those of several national and international studies (45,46).

The major virulence genes of *H. pylori*, including *cagA* and *vacA*, are polymorphic in different regions. The *cagA*-positive rate of *H. pylori* strains in Western countries has been reported as 50–60% (47,48), and the *cagA H. pylori* strain is closely related to the severity of the disease (49). In Western countries, patients infected with *cagA*-positive *H. pylori* strains have been shown to face an increased risk of peptic ulcers and gastric cancers than those infected with *cagA*-negative strains (50), and patients infected with multiple EPIYA-C segments *H. pylori* have been reported to have a higher gastric cancer risk than those infected with a single EPIYA-C segment strain (51). However, in Asia, particularly in China, Korea, and Japan, the positive rate of *cagA* was higher than 80–90% (52-55). The present study result is similar to that of Macau's by Pinto-Ribeiro *et al.* (56). A new study shows cagA-positive *H. pylori* strains can increase the risk of gastric ulcers and cancers, and the amounts of interleukins that are secreted during *H. pylori* infection are highly associated with the number and the variations within C-terminal EPIYA motifs; for instance, *H. pylori* strains with the EPIYA-D motif are prone to release higher amounts of interleukin-8 compared to other variations (57). In this study, 85 *H. pylori* strains were isolated and 74.1% were *cagA* East Asian type. Western strains (*cagA*-AB genotype) only appeared in patients with chronic non-atrophic gastritis. The gastric intraepithelial neoplasia and gastric cancer harbored both Asian strains (*cagA*-ABD genotype). However, it is notable that the Western type in this study only existed in the non-atrophic gastritis group, suggesting that the pathogenicity of the Western type is relatively weak and the risk of cancer is low.

VacA is another important virulence gene of *H. pylori* that has been studied worldwide. It also has geographical and ethnic differences, the *vacA s1c* genotype is common in East Asia, the *s1b* genotype is common in Spain, Latin America, and Poland, the *s1m1* genotype is common in Japan and South Korea (52,58), whereas the *m2* genotype is predominant in Taiwan and Myanmar (11). The result of this study shows that the predominant vacA genotype was *s1m1* and *s1m2* in Eastern China (59). The mosaic combination of *s*- and *m*-region allelic types has been shown to produce cytotoxins, and was associated with the bacterium's pathogenicity. In general, *s1m1* and *s1m2* strains produced high and moderate levels of toxins, respectively, whereas *s2m2* strains produced little or no toxin (22). The *vacA m1* strains have been associated with more significant gastric epithelial damage than *m2* strains (60). The *vacA* subtypes: *s1as1bm2*, *s2m2* and *m2*, *s1bm2* were significantly correlated to gastritis, whereas, subtypes *s1am1*, *s1am2*, *m1* were significantly associated with gastric and duodenal ulcers (12). A present study reveals a significant association between the strains carrying the *vacA m1* alleles and intestinal metaplasia in addition to gastric cancer (61). In this study, the *vacA* genotypes of the 85 *H. pylori* strains were diverse, but *s1c/m1b* (30.6%) and *s1c/m2* (41.2%) were the dominant genotypes and the most frequent combination in chronic non-atrophic gastritis. No correlation was found between *vacA* genotypes and gastric mucosa pathological changes; however, it showed the diversity and allelic combinations of *H. pylori* in different pathology.

The overall prevalence of *iceA* was 69.25% in China and 56.06% in other countries; a recent study showed that the prevalence of *iceA1* significantly increased the risk of peptic ulcer disease. In this study, the prevalence of *iceA1* was 64.7%, similar to that reported by Huang *et al.* (62). The presence of *iceA* was not associated with gastric cancer, but presence of *iceA1* was significantly associated with peptic ulcer, while the presence of *iceA2* was inversely associated

with peptic ulcer (63). Our results revealed no pathological differences regarding the of distribution of *H. pylori iceA* genotypes. The *iceA1* genotype was most commonly found genotype in chronic non-atrophic gastritis and chronic atrophic gastritis with intestinal metaplasia.

In addition to bacterial factors, mostly unknown host factors seem to influence the inflammatory response and the development of a more severe pathology. The response of T helper cells to *H. pylori* is generally thought to be the T-helper-1 (Th1) phenotype, leading to a cell-mediated immune response, whose important cytokines are interferon c (IFN-c), tumor necrosis factor A (TNF-a), and interleukin-1b (IL-1b). A study had shown that cytokine gene polymorphisms affect cytokine expression, gastric inflammation, and strain selection in *H. pylori* infection (64). A negative correlation between the presence of *cagA* and expression of programmed cell death protein-ligand 1 (PD-L1) mRNA in patients with gastritis, but not in ulcers. PD-L1 correlated positively with *vacA m1* and *vacA s1/m1*, whereas negatively with *vacA m2* and *vacA s1/m2* in patients with gastric ulcer. Furthermore, *vacA m1/m2* correlates negatively with programmed cell death protein-1 (PD-1) and PD-L1 in patients with gastritis, whereas positively with FOXP3 and interleukin-17 in gastritis and ulcer patients (65). Proliferation of gastric mucosa and the expression of mutated *p53* gene can be greatly increased by *H. pylori* during the development of gastric cancer, and *H. pylori* can induce apoptosis in the phase of metaplasia, but in the phase of dysplasia *H. pylori* can inhibit cellular apoptosis (66).

A primary limitation of this study was the small number of strains obtained. However, our results suggest that the strains causing more serious mucosal lesions and cancer risk in Guiyang are the East Asian strains. It is recommended that patients clinically infected with East Asian strains should be treated as soon as possible. With the promotion of early gastric cancer screening, it is necessary to increase the sample size, isolate *H. pylori* from the gastric mucosa of early cancer patients, continue virulence genotyping, and study the carcinogenic risk of EPIYA motif subtype of East Asian strains.

Conclusions

In conclusion, most patients with upper gastrointestinal diseases in this study had evidence of inflammation of the gastric mucosa, regardless of their *H. pylori* infection status. We found that the prevalence of *H. pylori* infection was related to the pathological type of gastric mucosal lesion. In patients with upper gastrointestinal diseases, endoscopy and biopsy of gastric mucosal lesions for pathological examination are recommended, regardless of the severity of the disease. The *cagA* East Asian type was the most common *cagA* genotype. The distribution of *cagA*, *vacA*, and *iceA* genotypes was not significantly correlated with the pathological type of gastric mucosa. Moreover, patients with gastric mucosal atrophy and intestinal metaplasia should undergo long-term follow-up observation with endoscopy to ensure timely treatment of the disease.

Acknowledgments

The authors would like to thank the Guiyang Hospital of Guizhou Aviation Industry and the Affiliated Cancer Hospital of Guizhou Medical University for providing us with an excellent data collection platform.
Funding: This work was supported by grants from the Research Foundation of The Affiliated Hospital of Guizhou Medical University (Nos. GYFYMF002 and GYFYMF001), and the Foundation of Key Laboratory of Microbiology and Parasitology of Education Department, Guizhou (No. QJJ [2022] 019).

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5553/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5553/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5553/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of the Affiliated Hospital of Guizhou Medical University (No. 2018-100) and informed consent was taken from all the patients. Guiyang Hospital of Guizhou Aviation Industry was informed and agreed the study.

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Cite this article as: Hu Y, Wang Y, Mi M, Deng Z, Zhu J, Liu Q, Chen X, Chen Z. Correlation analysis of gastric mucosal lesions with *Helicobacter pylori* infection and its virulence genotype in Guiyang, Guizhou province, China. Ann Transl Med 2022;10(24):1320. doi: 10.21037/atm-22-5553

