Infection and Drug Resistance

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ORIGINAL RESEARCH

Prevalence of *Helicobacter pylori* Antibiotic Resistance in Patients Enrolled in Guangzhou, China

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Purpose: *Helicobacter pylori* (*H. pylori*) infection is a high-risk factor for the occurrence of gastric cancer. The quadruple therapy has been widely used as the first-line treatment for *H. pylori* in China. However, the increasing resistance rate to antibiotics has become a major challenge in the treatment of *H. pylori*. Therefore, there is an urgent need for rapid and cost-effective detection of antibiotic resistance to different antibiotics. To evaluate the prevalence of *H. pylori* antibiotic resistance in Guangzhou and the diagnostic performance of DOB value of 13C UBT in predicting antibiotic resistance.

Patients and Methods: In this retrospective study, we collected data from 193 *H. pylori* culture-positive patients in Guangzhou on their DOB values and resistance to antibiotics. We analyzed the antibiotic resistance rate of commonly used antibiotics in quadruple therapy, and the diagnostic efficacy of DOB value was evaluated.

Results: The resistance rates of clarithromycin (CLA) and levofloxacin (LEV) were 46.1% and 44.0%, respectively. In the age group under 40, the resistance rate of LEV was lower than that of CLA. However, the diagnostic efficacy of DOB value was found to be low and it could not serve as an independent indicator for diagnosing resistance to CLA and LEV.

Conclusion: The high resistance rates of CLA and LEV in *H. pylori* patients in Guangzhou indicate the urgent need for effective detection methods. The DOB value is not a direct indicator of antibiotic resistance to CLA and LEV. Therefore, it is important to use a combination of diagnostic methods to accurately assess antibiotic resistance in *H. pylori* infection.

Keywords: *Helicobacter pylori*, urea breath test, DOB value, antibiotic resistance

Introduction

Helicobacter pylori (H. pylori) infection is a well-established class I carcinogen for the development of gastric cancer.[1] In China, the prevalence of *H. pylori* infection in the general population has been reported to be as high as 40–60%.[2] Eradication of *H. pylori* is an effective means to prevent gastric cancer.

Historically, triple therapy was the preferred treatment option due to its high eradication rates.[3] However, the emergence of drug resistance had led to the adoption of bismuth quadruple therapy.[4] Unfortunately, recent studies have shown an increase in drug resistance to quadruple therapy in China.[5] Drug resistance in *H. pylori* can result from various factors, including genetic mutations and inappropriate treatment for unrelated infections.[6] Therefore, drug susceptibility testing for *H. pylori* is crucial for successful eradication therapy.

Traditional methods for detecting drug resistance rely on *H. pylori* culture, which is time-consuming, expensive, and not suitable for large-scale applications.[7] Molecular methods offer a quicker and more cost-effective alternative for detecting drug resistance, however, the procedure involves a minimally invasive method of obtaining a small amount of gastric mucosa under gastroscopy.[8] The 13C-urea breath test (UBT) is a widely used clinical tool for diagnosing *H. pylori* infection, its non-invasive nature and ability to provide rapid results make it a valuable option for clinicians in diagnosing

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this condition. The UBT has been shown to have high sensitivity and specificity in detecting *H. pylori* infection, with reported value of 96% sensitivity and 93% specificity.[9] But its correlation with antibiotic resistance during eradication therapy has not been extensively studied. In addition to antibiotic resistance, factors such as patients' compliance and other variables may also contribute to treatment failure. In this study, we aimed to investigate the predictive value of Delta over baseline (DOB) values of UBT in determining the presence of drug resistance.

Materials and Methods

Study Design and Participants

This study included patients who underwent gastroscopy at The First Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine between June 2020 and December 2020. Inclusion criteria were as follows: (1) Male and female patients aged 18 to 85 years; (2) With gastrointestinal symptoms, such as abdominal pain, abdominal distension, acid reflux, belching, nausea, etc.; (3) Diagnosed with gastritis or duodenitis with erosion by gastroscopy; (4) Not having used antibiotics, bismuth, H2 receptor antagonists, or PPIs in the past 4 weeks; and (5) Agreeing to take gastric mucosal biopsy tissue samples for *H. pylori* culture and drug susceptibility testing and signing informed consent. Exclusion criteria were as follows: (1) Gastrointestinal emergencies, such as gastric perforation, peptic ulcer bleeding, hematemesis, acute pancreatitis, etc.; (2) Having received eradication therapy for H. pylori; (3) Having taken bismuth, PPI, H2 receptor antagonists, and antibacterial drugs in the past month; (4) Pregnancy and lactation; and (5) Having taken non-steroidal anti-inflammatory drugs or alcoholism or having other serious diseases that affect the results of this study, such as severe liver disease, heart disease, respiratory system diseases, etc. A total of 350 patients, including males and females aged between 18 and 85 years, with an average age of 51.4 ± 13.0 years, were initially considered for inclusion in this study. The deep mucosal tissue of the gastroscope was removed by a gastroenterologist under endoscope with sterile biopsy forceps within 5 cm from the pylorus on the lesser curvature of the antrum, placed in a centrifuge tube containing 0.9% NaCl solution, and transferred to the laboratory within 4 h. The study was approved by the Ethics Committee of The First Affiliated Hospital of Guangzhou University of Chinese Medicine (K-2022-12) and was conducted in accordance with the declaration of Helsinki.

13C-Urea Breath Test

All UBTs were carried out after more than 2 hours of fasting. A baseline breath sample was obtained, and then 75 mg of 13C-urea with citric acid (1.5 g) was administered with about 50 mL drinking water solution. Subsequently, another breath sample was collected 30 minutes after the administration of the test solution. The DOB values were obtained using a mass spectrometer to measure the difference in 13CO2 levels between the baseline sample and the 30-minute sample. A positive test result was defined as a difference exceeding 4.5 parts per 1000 of 13CO2. All the breath samples were analyzed using Beijing Huayuan Kangda 13C breath analyzer (HY 50), and the detection software was Beijing Huayuan Kangda 13C breath software.

H. pylori Isolation, Culture and Identification

Gastric mucosal tissues were fully ground by an automatic grinder to make tissue homogenates and inoculated into Columbia blood plates (5% sheep blood) and cultured in a microaerobic environment at 37°C in a three-gas incubator (5% O2, 10% CO2, 85% N2) for 96 h. Colonies with typical morphology and consistent bacterial morphology by smear microscopy were selected, and those with positive oxidase, catalase, and urease tests were identified as *H. pylori* strains and diluted with normal saline to 6×10^8 CFU/mL for subsequent drug susceptibility testing.

Drug Susceptibility Testing

Five antibiotics included in bismuth quadruple regimen were selected. According to the recommended protocol and interpretation criteria of American Association for Clinical Laboratory Standards (Wayne, PA, USA),[10] drug susceptibility testing was performed by agar dilution method with drug resistance cut-off point: the antibiotic solution was diluted into agar to the corresponding cut-off point concentration of the antibiotic, poured into the plate, inoculated with

bacterial suspension, and the bacterium was judged as resistant if there was bacterial growth on the plate. The cut-off point setting criteria of each antibiotic were: clarithromycin 1μg/mL, levofloxacin 2μg/mL, amoxicillin 2μg/mL, tetracycline hydrochloride 2μg/mL, and metronidazole 8μg/mL. All experiments were repeated at least twice.

Statistical Analysis

For comparison between two groups, if the data conformed to a normal distribution, data were presented as the mean±SD values, and statistical analyses were conducted using the unpaired *t*-test. And if not, a nonparametric method was used. A two-tailed $P < 0.05$ was considered significant. Kruskal–Wallis test was used to analyze among three or more groups. All statistical analyses were performed using SPSS20.

Results

A total of 350 patients were included in this study, and all cases underwent cultural examination for *H. pylori* using standard experimental methods. Of these cases, only 193 were successfully cultured from human gastric biopsy specimens. Drug susceptibility testing was then performed on these 193 cases, and the results are presented in Table 1. Notably, 46.1% (89/193) of cases were found to be resistant to clarithromycin (CLA), 44.0% (85/193) to levofloxacin (LEV), and 96.9% (187/193) to metronidazole (MET). In contrast, no resistance was observed for amoxicillin, tetracycline hydrochloride. Furthermore, 58 patients were found to be infected with strains resistant to both CLA and LEV, indicating multidrug resistance.

The results of our study indicate that age was not a significant factor in distinguishing between CLA sensitive and resistant populations. However, in the case of LEV, as presented in Table 2, the age of the resistant population was found to be higher than that of the susceptible population. To further investigate this relationship, we divided the 193 positive cases into different age groups, as shown in Table 2. Our analysis revealed that the prevalence of LEV resistance varied significantly across age groups, with the highest resistance prevalence observed in individuals aged 60 years or older, and the lowest prevalence observed in those under 40 years of age. Interestingly, we did not observe any significant differences in resistance prevalence between male and female groups for either CLA or LEV.

In this study, we conducted a C13 breath test to investigate the relationship between DOB values and resistance to two commonly used antibiotics, CLA and LEV. As presented in Table 3, our results showed that female participants had

Table 1 Characteristics of *H. pylori* Culture-Positive Cases

Characteristics	Number of Cases	Percentage of No. (%)
Gender		
Male	74	38.3
Female	119	61.7
Age(y)		
<40	39	20.2
40–59	103	53.4
≥60	51	26.4
CLA		
Susceptible	104	53.9
Resistance	89	46.1
LEV		
Susceptible	108	56.0
Resistance	85	44.0
MET		
Susceptible	6	3.1
Resistance	187	96.9
CLA+LEV		
All susceptible	77	39.9
All resistance	58	30.1

Abbreviations: CLA, clarithromycin; LEV, levofloxacin; MET, metronidazole.

Table 2 Resistance Prevalence of CLA and LEV Stratified by Age and Gender

Variables	CLA (No. of Cases)		LEV (No. of Cases)	
	Sensitive	Resistant	Sensitive	Resistant
Age (mean±SD)	51.34±13.23	51.08±12.22	49.51±13.5[a]	53.39±11.43[a]
<40	22(56.4)	17(43.6)	29(74.4)	10(25.6)
40–59	57(55.3)	46(44.7)	55(53.4)	48(46.6)
≥60	25(49.0)	26(51.0)	24(47.1)	27(52.9)[b]
Gender				
Male	40(54.1)	34(45.9)	41(55.4)	33(44.6)
Female	64(53.8)	55(46.2)	67(56.3)	52(43.7)

Notes: [a]$P<0.05$ in age between the LEV resistant and sensitive group; [b]$P<0.05$ in LEV resistance prevalence among different age groups.
Abbreviations: CLA, clarithromycin; LEV, levofloxacin.

Table 3 DOB Values Distribution According to Age and Gender

Variables	CLA (DOB Values)		LEV (DOB Values)		DOB Values
	Sensitive	Resistant	Sensitive	Resistant	
Age					
<40	41.62±20.44	40.55±13.94	42.22±19.36	37.83±10.07	41.13±17.40
40–59	45.42±24.45[a]	41.71±23.40[a]	39.82±19.19[a]	48.73±28.01[a]	43.96±23.94
≥60	37.10±19.71[&]	57.83±37.56[&]	39.45±22.52[&]	55.88±37.27[&]	48.41±32.18
Gender					
Male	34.48±14.69	39.57±21.63	35.48±14.87	38.22±21.71	36.65±17.96[#]
Female	48.16±25.48	52.30±32.24	43.60±22.30*	57.43±33.42*	50.11±28.76[#]

Notes: [a]The group that do not follow a normal distribution; [&]$P<0.05$ in the DOB values between the CLA/LEV sensitive and resistant group in the population aged ≥60 years; *$P<0.05$ in the DOB values between the LEV sensitive and resistant group in the female population; [#]$P<0.05$ in the DOB values between Male and Female.
Abbreviations: CLA, clarithromycin; LEV, levofloxacin.

significantly higher DOB values in the C13 breath test than male participants. Additionally, we found that DOB values were significantly higher in the LEV resistant group than in the sensitive group in females. Furthermore, we observed a statistically significant difference in DOB values between resistant and sensitive groups in both CLA and LEV in the age group ≥ 60 years, with DOB values being significantly higher in the resistant group than in the sensitive group. To determine whether DOB values could effectively predict CLA and LEV resistance in the age group ≥ 60 years, as well as

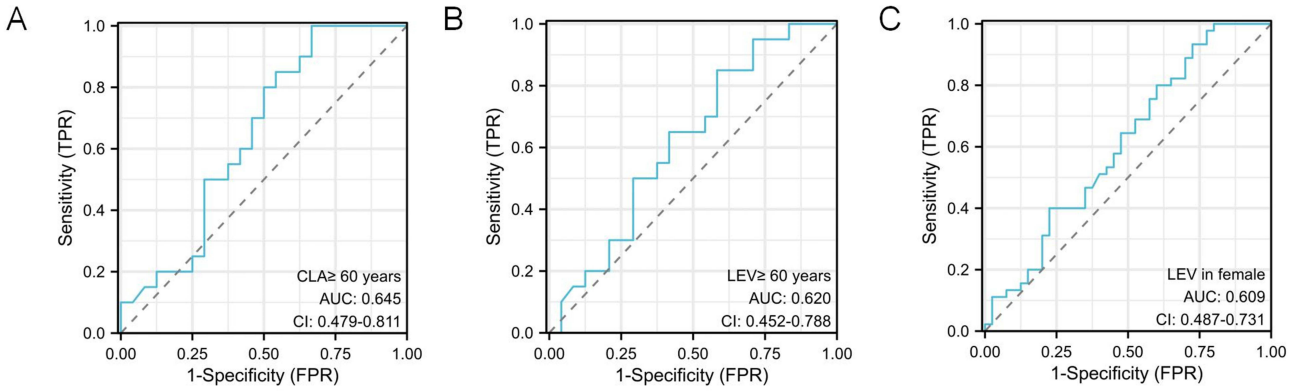

Figure 1 ROC curves were generated to evaluate the diagnostic performance of the DOB values. Specifically, DOB values were used to diagnose clarithromycin (CLA) resistance in the age group ≥ 60 years (**A**), levofloxacin (LEV) resistance in the age group ≥ 60 years (**B**), and LEV resistance in females (**C**). However, the diagnostic efficacy of the DOB values (Area Under the Curve, AUC) was found to be low in all three comparisons, with values of 0.645 for CLA resistance, 0.620 for LEV resistance in the age group ≥ 60 years, and 0.609 for LEV resistance in females.
Abbreviation: CI, confidence interval.

LEV resistance in females, we generated ROC curves to evaluate the diagnostic performance of DOB values in these three comparisons (Figure 1A–C). However, the diagnostic efficacy of the DOB values was 0.645, 0.620, and 0.609, respectively, which indicated low clinical diagnostic value.

Discussion

In China, bismuth quadruple therapy, which includes a proton pump inhibitor (PPI), bismuth, and two antibiotics, is the first-line treatment for *H. pylori* eradication.[11] The assessment of success and failure rates of H. pylori eradication in China holds significant clinical relevance. Recent studies have reported an average eradication rate of 81.3% in the bismuth quadruple therapy group and 71.3% in the triple therapy group.[12] These rates highlight the challenges associated with treating H. pylori infection in the Chinese population and emphasize the need to understand the factors contributing to treatment resistance. In our present study conducted in Guangzhou, China, we did not find any strains resistant to amoxicillin and tetracycline hydrochloride. However, the resistance prevalence of metronidazole was 96.9%, which is consistent with the findings of other researchers.[13] Notably, the resistance prevalence of CLA and LEV reached 46.1% and 44.0%, respectively. In a review published by Hu et al[14] the resistance prevalence of CLA in China ranged from 13.9% to 52.6%, while the prevalence of LEV resistance ranged from 12.6% to 54.8%. These findings highlight the urgent need for effective treatment strategies for *H. pylori* infections, particularly in cases of multidrug resistance.

In this study, we observed a significant difference in the prevalence of LEV resistance across various age groups. Our findings suggest that there is a trend towards increasing resistance to LEV with increasing age, with the lowest resistance prevalence observed in individuals aged less than 40 years. These results are consistent with a previous study conducted in northwest China.[15] Conversely, a study conducted in Chile reported an increase in the prevalence of LEV resistance with age in both men and women.[16] Notably, we did not observe a statistically significant difference in the prevalence of CLA resistance across different age groups. These findings highlight the importance of considering age as a potential risk factor for the development of LEV resistance. Further research is needed to elucidate the underlying mechanisms driving this age-related trend and to develop effective strategies for mitigating the emergence and spread of antibiotic resistance.

The C13 breath test is an important diagnostic method for Helicobacter pylori infection and is widely used worldwide. Consistent with a previous study,[17] we found that the DOB values of the C13 breath test were higher in females than in males. Additionally, we found that the DOB values of the LEV-resistant group in females were higher than those of the sensitive group. However, the results of the ROC curve demonstrated that the DOB values had only low diagnostic efficacy. The magnitude of DOB values can be influenced by various factors, such as H. pylori density, the grade of chronic gastric inflammation, and the presence of gastric mucosal atrophy. Regrettably, due to limitations in our study design, we did not evaluate these factors in relation to DOB values. Future studies should consider incorporating these variables. The C13 breath test is often used clinically after Helicobacter pylori eradication. A recent study found that a DOB value ≥20 before treatment was significantly correlated with H. pylori eradication failure.[18] However, few studies have investigated the diagnostic efficacy of DOB value in antibiotic resistance. Moreover, some studies have found no significant correlation between DOB value and antibiotic resistance.[19] In the present study, DOB values were higher in the LEV and CLA resistant groups than in the sensitive groups among individuals aged ≥60 years. However, it is important to note that DOB values only indicate the presence or absence of H. pylori infection, and should not be used as a substitute for susceptibility testing or molecular methods in determining levofloxacin or clarithromycin resistance.

Conclusion

In conclusion, our study revealed a high prevalence of resistance to CLA and LEV, and no resistance to amoxicillin and tetracycline hydrochloride in a sample of patients from Guangzhou, China. Interestingly, we found that the resistance prevalence of LEV was lower than that of CLA in patients under the age of 40. However, our results indicate that DOB values, which indicate the presence or absence of H. pylori infection, are not sufficient as a single diagnostic indicator for antibiotic resistance. Susceptibility testing or molecular methods remain the best choice for detecting antibiotic resistance.

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Disclosure

The authors declare no competing interests in this work.

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