Antibiotic Resistance of Helicobacter pylori in Nanjing: A Cross-Sectional Study

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1. Introduction

Since its discovery, *Helicobacter pylori* has become one of the most prevalent microorganisms globally, with an estimated worldwide infection rate of 44.3%. The burden is notably higher in developing countries (50.8%) compared to developed nations (34.7%) [1]. Beyond its established role in chronic active gastritis and peptic ulcer disease, *H. pylori* is now recognized as a primary risk factor for gastric cancer and mucosa-associated lymphoid tissue lymphoma. This is particularly significant in China, where gastric cancer ranks as the third most common malignancy in men and the second in women [2]. Consequently, *H. pylori* infection constitutes a major public health challenge[3]. The escalating threat of antimicrobial resistance further underscores the urgent need to improve eradication strategies[4, 5].

The Maastricht VI/Florence Consensus Report recommends routine antibiotic susceptibility testing to guide the selection of precise treatment regimens prior to *H. pylori* eradication[6]. Currently, only a limited number of antibiotics—including amoxicillin, clarithromycin, metronidazole, levofloxacin, tetracycline, and furazolidone—remain effective. However, their widespread use has accelerated the emergence of resistance[7, 8]. China, with its high burden of infection, faces a particularly complex landscape of antibiotic resistance [9].

Regimens containing clarithromycin and levofloxacin are effective strategies for both first-line and salvage therapy[10]. A meta-analysis highlighted that a 14-day levofloxacin-based sequential therapy is one of the most effective regimens worldwide[11]. However, levofloxacin susceptibility shows significant geographic variation. For instance, the average primary resistance rate in the Asia-Pacific region is approximately 18% [12], while in China it has risen to 34.21% [13].

Faced with continuously rising resistance rates to clarithromycin and levofloxacin, it is crucial to delineate two aspects: first, the dynamic trends in phenotypic resistance to these antibiotics in our region in recent years; and second, the characteristics of gene mutations associated with this resistance.

The increasingly severe resistance situation necessitates the establishment of continuous surveillance and the accumulation of local data. Our center has previously

published cross-sectional studies analyzing *H. pylori* resistance in Nanjing for the periods 2018–2021 and 2018–2023, focusing on the genotypic resistance of clarithromycin and levofloxacin. However, data on resistance trends for other antimicrobials, CYP2C19 gene polymorphisms, and their potential mechanisms remain limited. Therefore, this study systematically reviews the evolution of phenotypic and genotypic resistance rates of *H. pylori* to six commonly used antibiotics over the past seven years.

Beyond phenotypic analysis, this study investigates the genotypic resistance mechanisms for amoxicillin, clarithromycin, metronidazole, levofloxacin, tetracycline, and furazolidone. Resistance in *H. pylori* primarily results from mutations in genes encoding drug targets, leading to reduced efficacy. Evidence indicates that susceptibility can be effectively assessed by detecting mutations in the 23S rRNA gene (primarily point mutations A2143G, A2142G, and A2142C in domain V) for clarithromycin resistance; the gyrA gene for quinolone resistance; penicillin-binding protein genes for amoxicillin resistance; the 16S rRNA gene for tetracycline resistance; and the rdxA, frxA, and frxB genes for metronidazole resistance.

This study aims to provide an updated analysis of *H. pylori* resistance patterns in the Nanjing region, focusing on the current status of phenotypic and genotypic resistance to these six antimicrobial agents. The findings are expected to provide a scientific basis for the rapid diagnosis and personalized treatment of *H. pylori* infection.

2. Objectives

This project aims to analyze *H. pylori* resistance patterns in Nanjing through a retrospective review of medical records of outpatients who underwent *H. pylori* culture and genetic testing between 2018 and 2025. The focus is on characterizing the current status of phenotypic and genotypic resistance to amoxicillin, clarithromycin, metronidazole, levofloxacin, tetracycline, and furazolidone. The findings are intended to assist clinicians in the rapid diagnosis and individualized treatment of *H. pylori* infection.

3. Study Design, Methods, and Sample Collection

3.1. Study Design

This is a retrospective, single-center, cross-sectional epidemiological study. It involves a retrospective review of medical records to analyze *H. pylori* resistance patterns in Nanjing, with the goal of supporting rapid diagnosis and individualized treatment.

3.2. Methods

Patient records from the electronic medical record system and laboratory information system of Nanjing First Hospital were searched to identify all outpatients who underwent *H. pylori* culture and genetic testing at the gastroenterology clinic between January 1, 2018, and December 31, 2025, according to pre-defined inclusion and exclusion criteria.

3.3. Sample Collection and Requirements

Sample Type: This study retrospectively analyzes existing laboratory results from gastric mucosal biopsy samples stored in the hospital database. No new biological samples were collected for this research.

Sample Collection: All original samples were gastric mucosal tissues collected by clinicians during routine esophagogastroduodenoscopy for standard clinical care, following standard operating procedures.

3.4. Study Procedures and Technical Pathway

(1) Study Procedures:

1. **Study Initiation Meeting:** The principal investigator convened all research staff to review the study protocol, inclusion/exclusion criteria, data definitions, and extraction rules to ensure uniform understanding.

2. **Cohort Identification and Lock:** The data administrator retrieved and exported all 7,228 initial records of *H. pylori* testing from the hospital information system for the period from January 1, 2018, to December 31, 2025.

3. **Case Screening and Enrollment:** Two researchers independently screened the cases from the initial cohort according to the uniform inclusion/exclusion criteria. Discrepancies were resolved by a third senior researcher to finalize the list of patients

included in the analysis.

4. **Data Extraction:** Using a pre-designed electronic Case Report Form, the following information was extracted from the hospital's medical record and laboratory systems:

* **Basic Information:** Study ID, age, sex.

* **Clinical Information:** Endoscopic diagnosis, relevant medical history.

* **Laboratory Results:** *H. pylori* culture results, phenotypic antimicrobial susceptibility testing results for the six antibiotics, and mutation detection results for resistance-related genes.

5. **Data Cleaning and Database Lock:** Extracted data were cleaned to address missing values and logical errors. Key variables underwent independent double-data entry and verification. An anonymized research database suitable for analysis was formed as the final study cohort and locked without further changes.

6. **Statistical Analysis**

7. **Report Writing:** A comprehensive, standardized retrospective study report was written based on the locked data and analysis results.

(2) Technical Pathway

4. Participant Selection

4.1. Inclusion Criteria

Individuals meeting all of the following criteria were included:

(1) Patients who visited the gastroenterology outpatient clinic of Nanjing First Hospital and underwent gastroscopy with *H. pylori* culture and genetic testing between 2018 and 2025.

(2) Positive for *H. pylori* by at least one diagnostic method.

(3) Provision of general written informed consent for the use of gastroscopy and specimen data in research.

(4) Availability of complete medical records and laboratory test results.

4.2. Exclusion Criteria

Individuals meeting any of the following criteria were excluded:

(1) Withdrawal of informed consent; use of antibiotics, proton pump inhibitors,

H2-receptor antagonists, or bismuth agents within four weeks prior to gastroscopy.

(2) History of gastric surgery.

(3) Incomplete medical records or test results precluding evaluation.

(4) Other factors deemed by the investigator to render the participant unsuitable for inclusion.

4.3. Sample Size

Based on an initial search, a total of 7,228 records were identified within the specified timeframe. All cases meeting the inclusion and exclusion criteria were included in the analysis.

5. Alternative Diagnostic and Treatment Methods

For patients unsuitable for or unwilling to undergo gastroscopy, non-invasive diagnostic options such as the 13C-urea breath test or stool antigen test are clinically available.

In the absence of susceptibility testing, clinicians may empirically select eradication regimens based on regional resistance epidemiology or choose different combinations of salvage therapies based on the patient's prior medication history.

6. Testing Items and Time Points

Phenotypic Antimicrobial Susceptibility Testing: For isolated *H. pylori* strains, the minimum inhibitory concentration for amoxicillin, clarithromycin, metronidazole, levofloxacin, tetracycline, and furazolidone was determined using the agar dilution method or E-test. Resistance was interpreted according to CLSI or EUCAST standards.

Genotypic Resistance Detection: DNA from gastric tissue or isolates was analyzed using multiplex PCR or targeted sequencing to detect mutations in the following resistance-related genes:

* Clarithromycin: 23S rRNA gene
* Levofloxacin: gyrA gene
* Amoxicillin: PBP1A gene

* Tetracycline: 16S rRNA gene

* Metronidazole: rdxA, frxA genes

Testing Time Point: This study retrospectively analyzes the results of the above-mentioned tests that were completed at the time of initial diagnosis for all included cases. No new testing was arranged.

7. Efficacy Evaluation Criteria

As this is a retrospective surveillance study of resistance patterns and does not involve any therapeutic intervention, there are no efficacy outcomes. The primary outcome measures are the phenotypic resistance rates and genotypic mutation rates for each antibiotic.

8. Observation, Recording, and Management of Adverse Events

This is a retrospective data-based study that does not implement any new interventions. Therefore, it is not expected to directly cause any adverse events. The study only analyzes historical data and does not alter the patients' original diagnostic or treatment processes.

9. Quality Control and Quality Assurance

Data extraction and entry were performed independently by two researchers using a standardized Case Report Form, followed by cross-verification. Discrepancies were adjudicated by a third party. The reviewed laboratory data originated from the ISO-certified clinical laboratory of our hospital, where all operations followed standard operating procedures and included regular participation in external quality assessment schemes. All uses of retrospective data comply with hospital data security regulations, and ethical review exemption or approval has been obtained.

10. Data Safety Monitoring

During the study, all patient personal information was replaced with a unique study ID to ensure complete data anonymization. After study completion, the data will be

stored for at least five years to facilitate potential audits.

11. Statistical Analysis

Data analysis was performed using IBM SPSS Statistics (version 26.0). Percentages were used to describe the rates of *H. pylori* resistance to antibiotics. The Kappa consistency test was used to evaluate the agreement between antibiotic resistance phenotypes and genotypes. In statistical analysis, the Kappa coefficient ranges from -1 to 1. Typically, a Kappa value greater than 0.75 indicates substantial agreement.

12. Ethical Principles and Requirements

This clinical study was conducted in accordance with the Declaration of Helsinki and the Chinese National Health and Family Planning Commission's "Ethical Review of Biomedical Research Involving Humans." The principles of informed consent, privacy protection, research-related free services and compensation, risk control, protection of vulnerable subjects, and compensation for research-related injuries were strictly followed. The study protocol was approved by the ethics committee before initiation. Before enrollment, investigators were responsible for providing each participant or their legal guardian with a comprehensive explanation of the study's purpose, procedures, and potential risks, and for obtaining written informed consent. Participants were informed that their participation was entirely voluntary and that they could refuse to participate or withdraw from the study at any time without discrimination or retaliation, and that their medical care and rights would not be affected. The informed consent forms were retained as part of the clinical study documentation to ensure the protection of personal privacy and data confidentiality.

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