



Phenotypic Resistance Pattern of *Helicobacter pylori* Isolates to Clarithromycin, Amoxicillin and Metronidazole in Sana'a City, Yemen

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ABSTRACT

Background: *Helicobacter pylori* is a common bacterium infecting about half of the world's population, with higher rates in developing countries. Many strains of *H. pylori* are becoming antibiotic-resistant, making it increasingly difficult to treat infections. This resistance can lead to serious health issues, including an increased risk of gastric cancer. This study aimed to assess antibiotic resistance patterns among *H. pylori* isolates from Yemeni patients undergoing gastroendoscopy at the University of Science and Technology Hospital in Sana'a City, Yemen.

Methods: A prospective cross-sectional study was conducted on 116 isolates from gastric biopsy specimens. *H. pylori* was identified using rapid urease testing and histopathological examination of biopsy specimens. Positive samples were subsequently cultured on selective media to assess antibiotic susceptibility to clarithromycin (CLR), amoxicillin (AMX), and metronidazole (MTZ).

Results: Out of the 116 specimens analyzed, *H. pylori* was isolated from 62 samples (53.4%). Antibiotic susceptibility testing revealed resistance rates of 38.7% for CLR, 30.6% for AMX, and 87.1% for MTZ.

Conclusion: The rise in antibiotic resistance among *H. pylori* isolates from Yemeni patients emphasizes the critical need for regular resistance testing to inform treatment choices, improve patient care and minimize the risk of serious complications associated with *H. pylori* infections. Alternative regimens that exclude MTZ and CLR should be considered.

Keywords: *Helicobacter pylori* ▪ Clarithromycin ▪ Antibiotic resistance ▪ Yemen

1. Introduction

Helicobacter pylori is a urease-producing, Gram-negative bacterium that colonizes the gastric mucosa.⁽¹⁾ Approximately 50% of people worldwide are infected with *H. pylori*, with considerably higher prevalence in developing countries (85–95%); however, in developed countries, it is estimated to range from 25% to 30%.⁽²⁾ Approximately 80% of *H. pylori* infections remain latent for a long time with no

apparent symptoms, and people may not realize that they are infected.^(3,4) Such asymptomatic infections can lead to delayed treatment and raise the risk of complications associated with infection, including gastric ulcers, gastric cancer, mucosa-associated lymphoid tissue (MALT) lymphoma, and biliary tract cancer.^(3,4)

Treatment of *H. pylori* infections with antibiotics is the most efficient way to reduce ulcers and gastritis and to achieve successful healing following



an initial infection. Therefore, the selection of an effective bactericidal antibiotic is crucial.⁽⁵⁾ The standard triple therapy (STT), which typically includes clarithromycin (CLR), a proton pump inhibitor (PPI) and either amoxicillin (AMX) or metronidazole (MTZ), has been used as the classic first-line regimen for eradicating *H. pylori* infections for decades.⁽⁶⁾ However, *H. pylori* strains have become increasingly more resistant to CLR in recent years, decreasing the effectiveness of most commonly prescribed antibiotic regimens.⁽⁷⁾ In recent years, the success rate of first-line therapy has declined to below the recommended threshold of 80%.⁽⁸⁾ Treatment failure is thought to be primarily caused by non-compliance and the establishment of antibiotic-resistant strains of *H. pylori*. The STT is currently recommended only in regions where CLR resistance remains below 15–20%.⁽⁸⁾ Several studies have reported varying but high rates of antibiotic resistance among *H. pylori* isolates, particularly to CLR.^(9–13)

In Yemen, despite the high prevalence of *H. pylori* infection (>80%),^(14,15) there is a lack of published data on phenotypic antibiotic resistance among patients undergoing gastroendoscopy. Therefore, this study aimed to assess phenotypic resistance to CLR, AMX, and MTZ among *H. pylori* isolates from these patients at a tertiary care hospital in Sana'a City, Yemen.

2. Methods

2.1. Study design, specimens and setting

A prospective cross-sectional study was conducted from October 2023 to May 2024 to isolate *H. pylori* and determine its antibiotic susceptibility patterns among patients undergoing upper gastroendoscopy at the University of Science and Technology Hospital in Sana'a. Biopsy specimens were obtained from patients presenting with symptoms suggestive of gastritis. The sample size was determined using Epi Info™ software, version 7 (CDC, Atlanta, Georgia, USA), assuming a 95% confidence level, an expected

CLR resistance prevalence of 6.7%,⁽¹⁶⁾ and a 5% margin of error. Based on these parameters, the minimum required sample size was 96 patients. To compensate for possible dropouts, the sample size was increased to 116 patients.

Patients who had used proton pump inhibitors, antibiotics, or non-steroidal anti-inflammatory drugs within the preceding 4 weeks, those younger than 18 years, and pregnant women were excluded from the study. During endoscopy, gastric biopsy specimens were obtained from the antrum and used for *H. pylori* diagnosis by rapid urease test (HelicotecUT® Plus, Strong BioTech Corp., Taiwan) and histopathological examination.

2.2. Gastric biopsy culture and identification of *H. pylori*

Gastric biopsy specimens were gently crushed with sterile forceps and, using a sterile loop, inoculated directly onto *Campylobacter* agar (Figure 1) supplemented with 10% (v/v) horse blood and rehydrated *Campylobacter* Selective Supplement (trimethoprim 2.5 mg/500 mL, cefsulodin 2.5 mg/500 mL, vancomycin 5 mg/500 mL, and amphotericin B 2.5 mg/500 mL). Plates were incubated in a candle jar at 37 °C for 5–7 days. Suspected colonies were identified as *H. pylori* by positive urease, catalase, and oxidase tests.



Figure 1: Isolation of *H. pylori* on *Campylobacter* agar



2.3. Antibiotic susceptibility testing

Antibiotic susceptibility was determined for *H. pylori* isolates using the Kirby-Bauer disk diffusion method. The inoculum opacity was adjusted to a 0.5 McFarland standard, and *H. pylori* isolates were inoculated onto Mueller-Hinton agar supplemented with 10% (v/v) horse blood. The following antibiotic discs (HiMedia Laboratories Pvt. Ltd., Mumbai, India) were used: CLR 15 µg, AMX 10 µg, and MTZ 5 µg. Inhibition zone diameter breakpoints for resistance were defined as ≤13 mm for CLR, <14 mm for AMX, and <16 mm for MTZ.^(17–19)

2.4. Data analysis

Data were analyzed using IBM SPSS Statistics software, version 27.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were presented as frequencies and percentages.

3. Results

3.1. Resistance pattern of *H. pylori* isolates

Overall, *H. pylori* isolates were successfully obtained from 62 out of 116 biopsy specimens, yielding a culture success rate of 53.4%. Antibiotic susceptibility testing of these isolates revealed resistance rates of 38.7% to CLR, 30.6% to AMX, and 87.1% to MTZ (Figure 2).

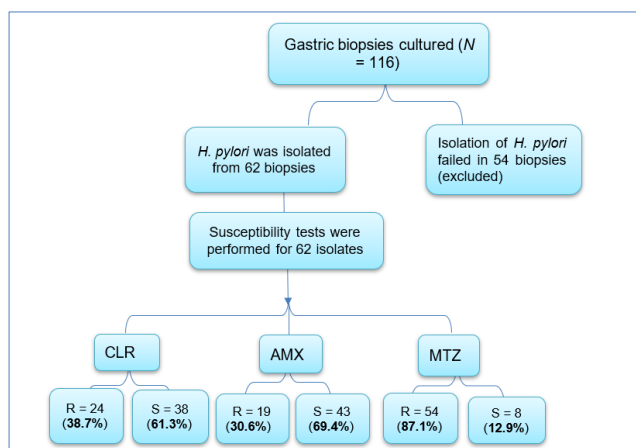


Figure 2: Flow diagram of *H. pylori* isolation, antibiotic susceptibility testing, and antibiotic resistance pattern

CLR, clarithromycin; AMX, amoxicillin; MTZ, metronidazole; R, resistant; S, sensitive

4. Discussion

H. pylori is a common opportunistic pathogen most frequently associated with peptic ulcers, gastritis, gastric cancer, and other gastrointestinal disorders.⁽²⁰⁾ The first-line treatment for *H. pylori* infection has traditionally been the STT, which involves taking two antibiotics in addition to a PPI and is still effective in areas with low drug resistance. However, increasing resistance to CLR, which is the key antibiotic in this regimen, has led to a marked decline in the effectiveness of this regimen, with eradication rates of the STT dropping from 90% to <70%.^(1,12,21,22)

Culture-based methods remain the reference standard for determining antibiotic susceptibility and for longitudinal monitoring of resistance trends.⁽²³⁾ In the present study, *H. pylori* was successfully isolated from slightly more than half of the gastric biopsy specimens, a rate comparable to that reported in Iraq (47.8%).⁽¹⁰⁾ In contrast, a much higher isolation rate of 91.8% was documented in China,⁽⁹⁾ and a substantially lower rate of 13.7% was reported in India.⁽²⁴⁾ These variations in culture yield could be attributed to challenges in *H. pylori* culturing, including the requirement for specialized laboratory equipment, reagents and media, strict microaerophilic growth conditions, and skilled professionals. All of these are linked to high costs and frequently restrict the broad application of this approach in clinical practice. Moreover, at least two biopsies may be required, as bacterial load can vary considerably between specimens as a result of the patchy distribution of *H. pylori* within the gastric mucosa and the possibility of low levels of colonization in individual biopsies.^(23,25)

Using the disc diffusion method, resistance rates of *H. pylori* isolates in this study were 38.7% for CLR, 30.6% for AMX, and 87.1% for MTZ, indicating a particularly high level of resistance to MTZ compared with the other two agents. When compared with



data from neighboring and regional countries, variations in resistance patterns are evident. In Saudi Arabia, a 2015 study using the E-test found lower resistance rates of 23.3% for CLR, 14.8% for AMX, and 48.5% for MTZ.⁽²⁶⁾ In Oman, a more recent study reported resistance rates of 33.3% for CLR, 66.7% for AMX, and 100% for MTZ.⁽²⁷⁾ On the other hand, resistance rates of 69.1% for CLR and 72.7% for MTZ were reported in Iraq, using the E-test.⁽¹⁰⁾ Recently, the disc diffusion method showed resistance rates of 28.7% for CLR, 83.2% for MTZ, and 67.6% for AMX.⁽¹³⁾ A 2023 Turkish study using an antibiotic gradient test reported resistance rates of 28.5% for CLR and 44.8% for MTZ, with no resistance detected to AMX.⁽¹²⁾ In China (2023), E-test data showed resistance rates of 38.9% for CLR, 57.3% for MTZ, and only 2.6% for AMX,⁽⁹⁾ while in Korea (2023), CLR resistance of 18.6% was reported using the agar dilution method.⁽¹¹⁾ Such wide variability across countries likely reflects differences in local antibiotic use and prescribing habits, which influence selective pressure on *H. pylori* strains. In addition, using different testing methods, such as the disc diffusion versus the E-test or agar dilution, can yield slightly different results and may contribute to these variations.⁽²⁸⁾

The consistently high MTZ resistance observed in this study and many other studies could be partly attributed to the recurrent and broad clinical use of MTZ for treating parasitic, dental, and gynecological infections. This widespread exposure exerts strong selective pressure, allowing resistant *H. pylori* strains to emerge and persist.⁽²⁹⁾ In contrast, AMX resistance remains relatively uncommon worldwide, which may be due to the low prevalence of β -lactamase-mediated resistance mechanisms in *H. pylori* and the limited overuse of this antibiotic for gastric infections.⁽¹⁾ CLR resistance, however, has become a growing global concern, largely attributed to the overuse of macrolides in respiratory tract infections.⁽³⁰⁾ These findings emphasize the need for

continuous regional surveillance and the adoption of locally tailored *H. pylori* treatment strategies that account for evolving antibiotic patterns.⁽¹³⁾

This study has a number of limitations that must be considered. First, the study was conducted in a single hospital, which may limit the generalizability of findings to other regions of Yemen or to the community patterns of *H. pylori* resistance. Second, the low isolation success rate of *H. pylori* isolation may reduce the precision of the estimated resistance estimates. Finally, antibiotic sensitivity was determined using the disc diffusion method rather than minimum inhibitory concentration (MIC)-based methods (E-test or agar dilution), which are considered more precise reference methods for *H. pylori*. Therefore, this methodological difference may affect direct comparability with other studies. Based on these limitations, future multicenter studies with larger sample sizes are recommended, combining culture-based susceptibility testing with MIC determination and molecular analysis of resistance genes.

5. Conclusion

The rise in antibiotic resistance among *H. pylori* isolates from Yemeni patients emphasizes the critical need for regular resistance testing to inform treatment choices, improve patient care, and minimize the risk of serious complications associated with *H. pylori* infections. Alternative regimens that exclude MTZ and CLR should be considered.

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Ethical approval

Ethical approval was obtained from the Research Ethics Committee of the University of Science and Technology (Ethical clearance No.: 1445/006/UREC/UST). In addition, permission to conduct the study was obtained from the administration of University of Science and Technology Hospital. Written informed consent was obtained from each participant, and all collected data were kept private and confidential.

Conflict of Interest

The authors declare no conflict of interest associated with this article.

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