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Antibiotic Susceptibility Profiling of *Helicobacter pylori* in Iraq

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Abstract :

Helicobacter pylori (H. pylori), a pathogenic bacterium implicated in various gastric disorders, were comprehensively investigated in a population in Iraq. Rapid urease testing identified 42.15% of samples as positive, with subsequent culturing confirming characteristics typical of *H. pylori*. Molecular confirmation through 16s rRNA gene, PCR exhibited an eighty percent positivity rate, supporting accurate species identification. Antibiotic sensitivity testing revealed resistance to metronidazole, with notable sensitivity to levofloxacin and moxifloxacin. However, a concerning 85.7% of isolates demonstrated multi-drug resistance.

Key words: *Helicobacter pylori*, Antibiotics, Resistance, Treatment.

فحص الحساسية الدوائية لبكتريا الملوية البوابية في العراق

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مستخلص

تم فحص بكتريا الملوية البوابية ، وهي جرثومة مسببة للعديد من الاضطرابات المعوية المختلفة ، في مجموعة سكانية في العراق. حدد اختبار اليوريا السريع 42.15٪ من العينات على انها ايجابية ، مع زراعة لاحقة تؤكد الخصائص النموذجية لهذه البكتريا. التأكيد الجزيئي للعينات المزروعة اثبت معدل ايجابية 80٪ ، مما يدعم التشخيص الدقيق للانواع. كشف اختبار حساسية المضادات الحيوية عن المقاومة الكلية للميترونيدازول ، مع حساسية ملحوظة لليفوفلوكساسين و الموكسيفلوكساسين. ومع ذلك، اظهرت 85.7٪ من العزلات مقاومة متعددة الأدوية.

الكلمات المفتاحية : الملوية البوابية ، المضادات الحيوية ، المقاومة ، العلاج .

Introduction

Helicobacter pylori (H. pylori) infection is the major cause of gastritis, peptic ulcers in over-half of world population, and considered as a major important risk of gastric cancer (Mégraud *et al.*, 2023). The bacterium is curved bacillus, appear as red in the Gram stain; catalase, oxidase and urease positive, motile by multi-unipolar flagella, fastidious and prefers microaerophilic conditions (Adinortey *et al.*, 2018). In 2022 Ferreira *et al.* reported that central Asia and Africa have the highest prevalence of *H. pylori* infections (>79%), while Oceania had the lowest rate (24.4%). In Iraq, it was found that the prevalence of *H. pylori* in Baghdad 74.2%, Diyala government 75.2%, and in Erbil city it was 53.5% as reported by Al-Jumaili *et al.* (2022), Abdul-Rahman *et al.* (2022) and Majeed and Khoshnaw (2020) respectively.

Many antibiotic treatments are used for the treatment of *H. pylori*, and studies show that the number of strains resistant to antibiotics is increasing rapidly (Öztekin *et al.*, 2021). Mabeku *et al.* (2019) mentioned that combined therapy is used to eradicate *H. pylori* infection, triple therapy, including two antibiotics, amoxicillin and clarithromycin, and a proton pump inhibitor given for a week has been recommended as the treatment of choice at several consensus conferences.

Materials and methods

A total of 102 gastric biopsy samples were collected from patients suffer from gastric pain, burning, dyspepsia, weight loss or dysphagia. Three biopsies from different gastric regions (two from gastric antrum and one from the fundus) were taken from each patient; two were for the rapid urease test (RUT) and one for the culturing process. The samples transported to the laboratory within 8 hours in stuart transport media at cooling conditions, as it mentioned by Soltesz *et al.* (1992) as the optimal media for transporting of *H.pylori* specimens.

The RUT-positive biopsies were homogenized manually with 500µl normal saline and cultured on Skirrow agar based on columbia blood agar with in anaerobic conditions, incubated at 37°C for 3-7 days before confirming culture result. All positive cultures were subjected to Gram stain and biochemical tests.

The positive results were tested by PCR for confirmation of diagno-

sis through presence of specific 16s rRNA gene using forward primer 5'-TTGGAGGGCTTAGTCTCT-3' and reverse primer 5'- AAGATTGGCTCCACTTCACA -3' that designed by Salman *et al.* (2021). Positive samples were tested for the antibiotic susceptibility by disk diffusion method using Clarithromycin 15 µg (CL) , Metronidazole 5 µg (MET), Levofloxacin 5 µg (LE), Tetracyclin 30 µg (TE), Amoxycillin 30 µg (AX) were selected based on the treatment protocol against *H. pylori* infection according to *H. pylori* treatment guideline (Aumpan *et al.*, 2023), and Moxifloxacin 5 µg (MO) was selected out of the treatment guideline and based on the local physicians use.

The antibiotic susceptibility test breakpoints were determined based on past studies used the same antibiotics, MET antibiotic resistance breakpoint was determined as 16 mm by (CHAVES *et al.*, 1999), while breakpoints for AX and TET antibiotics were selected as 25 mm and 21mm for CL as mentioned by Lang and García (2004). For LE antibiotic it was calculated by Tang *et al.* (2020) as 29 mm, while the MO breakpoints were selected as 18 mm based on standards for *Haemophilus influenza* from (Clinical and Laboratory Standards Instituite, 2023).

Results

Rapid Urease test was the first step of diagnosis of *H. pylori* and revealed 43/102 (42.15%) positive samples. A total of 26/43 (60%) were cultured. The colonies appeared as white-grey pinhead small colonies on Columbia blood agar (Figure 1). The bacterium was Gram negative, small curved bacillus, biochemically it was urease, catalase and oxidase positive as shown in Figure (2) and Figure (3) respectively.

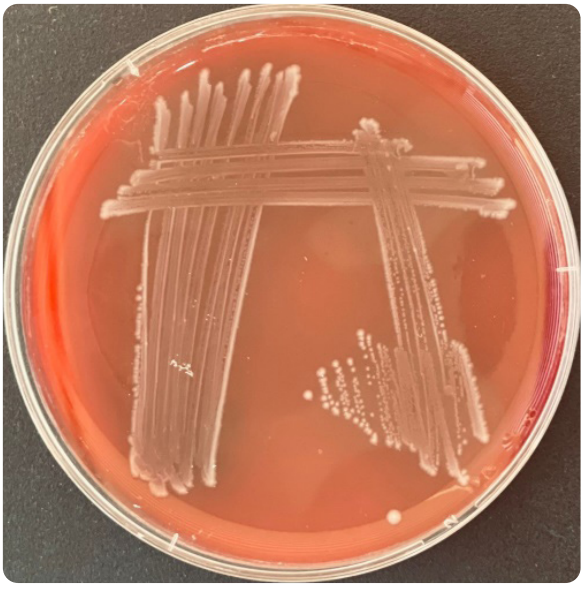


Figure (1): *Helicobacter pylori* colonies on Columbia blood agar appeared as white-grey pinhead small colonies

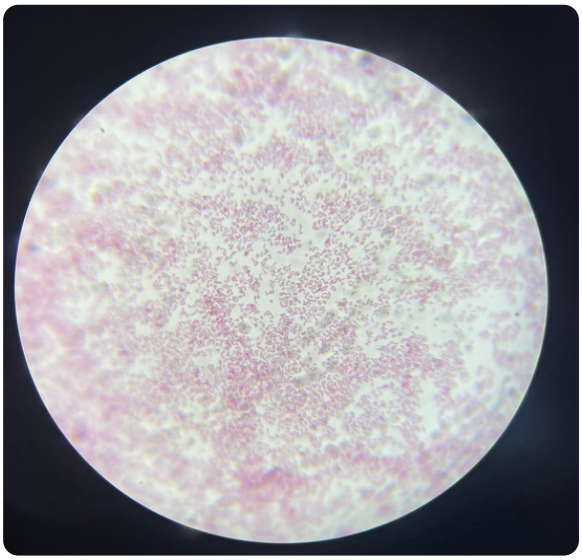
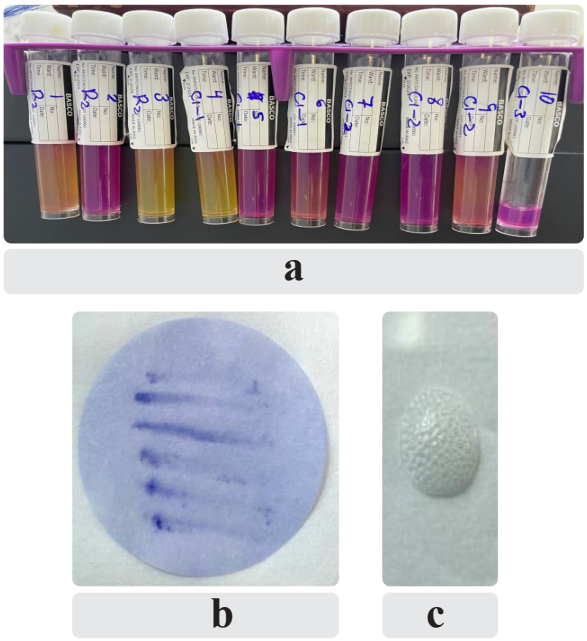


Figure (2): *Helicobacter pylori;* gram negative curved rods



a

b **c**

Figure (3): Biochemical tests: a- Urease test; b- Oxidase test; c- Catalase test

The molecular confirmation by conventional PCR using specific primer 16s rRNA confirmed the diagnosis of 21/26 *H. pylori* cultured samples and the amplified bands appear at 495 bp.

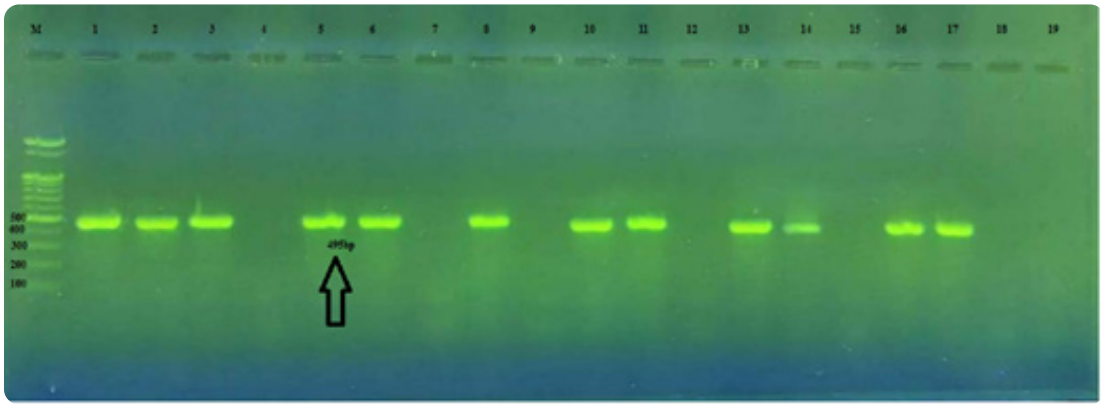


Figure (3): Electrophoresis of 16s rRNA gene (495 bp) on 1.5% agarose gel for 90 mins at 80V/cm in presence of DNA ladder marker (M).

Cultured and confirmed samples were tested for antibiotic sensitivity on Muller-Hinton agar and the outcome clarified that all samples (100%) were resist for metronidazole, sensitive to levofloxacin and moxifloxacin , while

the results for other antibiotics were; 10/21 (47.6%) resist to clarithromycin, 15/21 (71.4%) resist to tetracycline, and 13/21 (61.9%) were resist to amoxicillin, it was found that 18/21 (85.7%) of isolates were multi-drug resistant, 11/21 (52.3%) showed dual resistance (including 8 to MET+TET and 3 to MET+CL). Among the strains with triple resistance, 7/21 (33.3%) was resist to MET+TET+CL as showed in Table (1).

Table (1) Resistance patterns among the 21 *H. pylori* isolates

Resistance Pattern	n %
None Resist	0 %
Mono Resistance	
MET	14.2%
Dual Resistance	
MET+ TET	38%
MET+CL	14.2%
Triple Resistance	
MET+TET+CL	33.3%

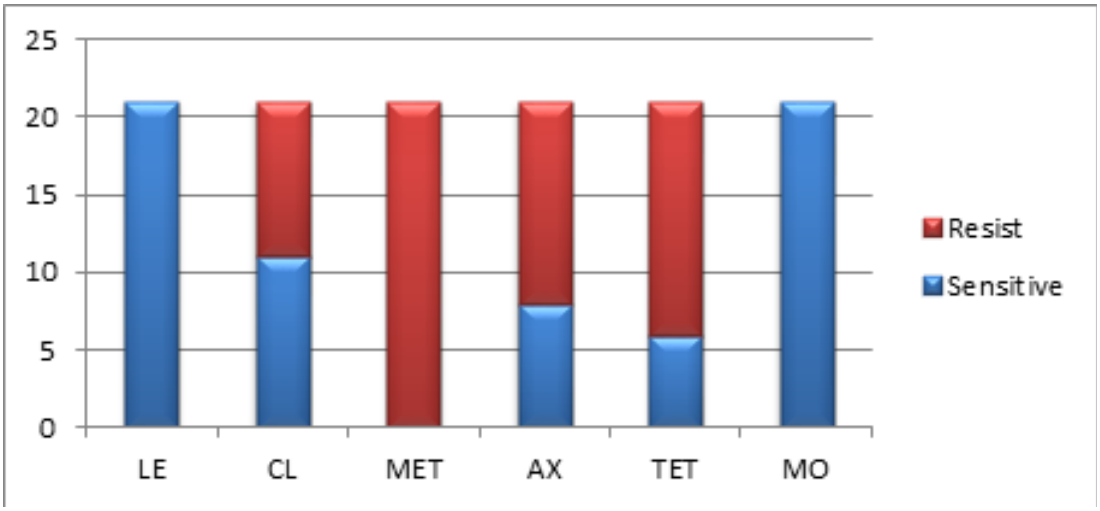


Figure (4): The overall sensitive and resistance rates of the 21 *H. pylori* isolates against six antibiotics.

Discussion

In the last years, the resist of *H. pylori* increased towards antibiotics, leading to the treatment failure and causes more severe infections may lead to gastric cancer. The susceptibility test

very important step should be carried out which potentially leading to more effective treatment and improve clinical outcome.

When comparing the result of the present study with other local studies in Table (2), it was clear that *H. pylori* have been fully resist against metronidazole, while amoxicillin and clarithromycin resistance have been increased in comparison with the studies in 2014 and 2022; tetracycline has a close resistance rate from 2022 but both have been increased in comparison with 2014.

In the other hand, there was stability in the sensitivity against levofloxacin; this result is supported by the two studies mentioned, Saeed *et al.* (2014) and Saber and Ali (2022). The last antibiotic which is used by local physicians showed efficiency against *H. pylori* with a rate of 100% sensitive; based on this result it was suggested and recommended to use it as an effective antibiotic against *H. pylori* infection.

Table (1): Comparison of present results of *H. pylori* antibiotic resistance with previous studies in Iraq region

Antibiotic	Present Study	Saber and Ali (2022)	Saeed *et al.* (2014)
Levofloxacin	0%	0%	0%
Metronidazole	100%	100%	53.3%
Tetracycline	71.4%	80%	16.6%
Clarithromycin	47.6%	30%	10%
Amoxicillin	61.9%	40%	6.7%

Several studies conducted with the mechanism of bacteria to fight antibiotics, in general, mechanisms can be grouped in three classes as mentioned by Zanotti and Cendron (2019) : (i) mutations of specific key residues in the inhibitor-binding protein, (ii) regulation of efflux systems or membrane permeability to minimize antibiotic uptake, and (iii) additional intricate indirect effects. A study by Huang *et al.* (2023) reported that metronidazole resistance mechanisms of *H. pylori* include disturbed ezymes, drug efflux, biofilm formation and chemotaxis.

Clarithromycin's action involves binding to *H. pylori's* 50S bacterial ribosomal subunit, inhibiting protein synthesis and causing a bacteriostatic effect; its resistance relies on specific

mutations in the 23S rRNA peptidyl transferase region, negatively impacting clarithromycin's affinity for the bacterial ribosome; additionally, an efflux pump mechanism effectively expels clarithromycin, particularly in 23S rRNA mutant strains, indicating a synergistic interaction with mutational resistance (Francesco *et al.*, 2011; Hirata *et al.,* 2012).

In a recent study by Matta *et al.,* (2023) on amoxicillin, it was found that the antibiotic binds to penicillin-binding proteins (PBPs) in the bacterial periplasm, affecting transpeptidase activity crucial for cross-linking peptidoglycan molecules, ultimately hindering peptidoglycan synthesis and impeding *H. pylori* growth. According to Tshibangu-Kabamba and Yamaoka (2021), amoxicillin resistance in *H. pylori* often arises from mutations that reduce the binding of the antibiotic to penicillin-binding protein PBP1A, and mutations in PBP2 or PBP3 may further contribute to increased resistance.

Conclusion

The increasing resistance of *H. pylori* to antibiotics poses a significant challenge, leading to treatment failures and a heightened risk of severe infections, including the potential development of gastric cancer. The importance of conducting susceptibility tests cannot be overstated, as they play a crucial role in tailoring more effective treatments and improving clinical outcomes.

The comparative analysis of the current study with previous research underscores the alarming trend of *H. pylori* resistance, particularly against metronidazole, amoxicillin, and clarithromycin. Levofloxacin remains a reliable option with consistent sensitivity, suggesting its continued efficacy *against H. pylori* infections. The recommendation of the last antibiotic, boasting a 100% sensitivity rate, emphasizes its potential as an effective treatment strategy.

Exploration of bacterial resistance mechanisms reveals the complexity of *H. pylori'*s ability to counteract antibiotics, encompassing mutations in key residues, regulation of efflux systems, and additional intricate indirect effects. Specific insights into clarithromycin and amoxicillin mechanisms further highlight the importance of understanding how these antibiotics interact with *H. pylori*, guiding the development of targeted strategies to combat antibiotic

resistance in this bacterium. Overall, addressing antibiotic resistance in *H. pylori* necessitates a comprehensive and multidimensional approach to ensure the effectiveness of future therapeutic interventions.

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