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Phenotypic detection of some drug resistance and virulence factors among *Helicobacter pylori* isolated from clinical sources in Iraq

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Helicobacter pylori colonizes the gastrointestinal tract and can produce diseases leading to gastric problems ranging from gastritis to peptic ulcers or macroadenomas. In these cases, treatment is critical. However, antibiotic resistance could potentially reduce the antibiotic efficacy leading to disease progression. The present study aimed to characterize the resistance profile of commonly used antibiotics in our community, including metronidazole, clarithromycin, amoxicillin, and tetracycline. *Helicobacter pylori* were cultured and characterised for identification and virulence factor production. For *H. pylori* isolates from individuals with gastrointestinal illnesses, a set of drugs, including clarithromycin, tetracycline, amoxicillin and metronidazole, was used in the disk diffusion and agar dilution procedures. 25 individuals with gastrointestinal illnesses out of 100 patients tested positive for *H. pylori* isolates. After that, an antibiotic sensitivity test was performed, and the results showed that 9/25 (36%) of the isolates were resistant to metronidazole (MICs ≥ 6 μg/L), while 7/25 (28%) were resistant to amoxicillin (MICs ≤ 1 μg/mL), while resistance rates were 6/25 (24%) to tetracycline and 3/25 (12%) to clarithromycin (MICs ≤ 4 μg/mL). The present study confirmed that resistance exists with metronidazole being the highest while clarithromycin, amoxicillin, and tetracycline have lower resistance than metronidazole.

Keywords: *Helicobacter pylori*; gastritis; metronidazole; clarithromycin; amoxicillin; tetracycline.

Introduction

One of the common causative agents of gastrointestinal tract (GIT) infections is *Helicobacter pylori* (Ibrahim, 2024). This Gram-negative bacterium is a spiral microaerophilic bacterium which settles freely on the gastric mucous surface with negligible penetration capacity (Semino-Mora et al., 2003), although, slight adherence to the gastric epithelial apex has been demonstrated (Bashir & Khan, 2023), hence it can potentially induce mucosal breach (Semino-Mora et al., 2003). One of the most frequent causes of numerous infectious disorders is thought to be *H. pylori*. This bacterium's infection is estimated to be 70% worldwide (Parra-Sepúlveda et al., 2019). Many times, *H. pylori* infections are asymptomatic and do not cause stomach adenocarcinoma (Semino-Mora et al., 2003), mucosa-associated lymphoid tissue (MALT) lymphoma (Hu et al., 2016), or ulcers related to the infection (Ibrahim, 2024).

To prevent gastric and duodenal diseases and their associated complications, the treatment aims to eradicate symptomatic *H. pylori* infection through the use of multiple therapeutic regimens as well as antimicrobial agents. *H.pylori* has a high ability to resist antimicrobials (Marshall & Warren, 1984; Bashir & Khan, 2023). The combination of tetracycline, metronidazole, amoxicillin, and clarithromycin with a proton pump inhibitor (PPI) is one of the most effective ways to eradicate *H. pylori* infection (Miendje Deyi et al., 2011; Ang et al., 2016). The rates of resistance to antibiotics by *H. pylori* vary depending on the nature and method of use of the antibiotic because improper and careless use has caused the establishment of many drug-resistant strains in addition to the occurrence of genetic mutations and the acquisition of genes that encode for many antibiotics (Liang et al., 2020). The present study is designed to characterize the antibiotic resistance to the commonly used antibiotics against *H. pylori*, including metronidazole, clarithromycin, amoxicillin, and tetracycline.

Materials and methods

Specimens collection. The collected stool samples (n = 100, age 25–50 years) from patients presenting with gastric upset were used during the period February to April 2024 in Medical City Hospital (Baghdad, Iraq).

Exclusion criteria. Patients presented to the gastroenterology department with gastric upsets were included in the present study. Patients with any chronic diseases or using chronic medication were excluded. Patients aged less than 18 or older than 50 years were excluded. Overweight and obese patients were also excluded. Patients with gastric upsets associated with diarrhoea were excluded. Pregnant and lactating women were excluded.

Isolation and identification of H. pylori. The collected specimens were directly cultured on Columbia Agar media containing 7–10% blood suitable for *H. pylori* culture. Once *H. pylori* was isolated, they were cultured in a selective and ideal medium for *H. pylori* and brain heart infusion, to be used for subsequent experimentations.

Culture of H. pylori. The Petri dishes which showed growth (25%) were further tested to confirm *H. pylori* isolates by conducting biochemical tests, including catalase, oxidase, urease, methyl red, indol test, and citrate utilization.

Antibiotic susceptibility testing. All positive isolates of *H. pylori* were tested for bacterial susceptibility to antibiotics using the Kirby-Bauer method for a group of antibiotics (ciprofloxacin, clarithromycin, tetracycline, amoxicillin, and metronidazole) and minimum inhibitory concentrations (MICs) on Muller-Hinton Agar containing 5% sheep blood using the agar dilution method.

In the present study, antibiotics were added to the medium at multiple concentrations, varying from 0.016 to 256 μg/mL, which were treated by cooling them to 45 °C. Suspensions of *H. pylori* were prepared, and then 3 μL of these suspensions and 2 McFarland standards were inoculated to Mueller-Hinton Agar plates that contained different concentrations of antibiotics. The MIC of each antibiotic

was then ascertained after the culture plates were incubated at 37 °C for three to five days in a microaerobic environment (5% O_2, 10% CO_2, 85% N_2). Resistance to tetracycline was ≤ 4 and clarithromycin ≤ 4 µg/mL, while amoxicillin and metronidazole were ≤ 1 and >12 µg/mL, respectively.

Phenotypic detection of some virulence factors among H. pylori. The ability of *H. pylori* isolates to produce virulence factors such as protease and lipase enzymes, in addition to biofilm production, increases their ability to cause pathogenicity and resistance to various antibiotics. Positive *H. pylori* isolates were cultured on Soyabean Casein Digest Agar plates supplemented with 1% Tween 80 to determine the ability of the bacteria to produce lipase enzyme through the appearance of a turbid halo around the inocula. While the isolates of *H. pylori* were grown on Muller-Hinton Agar containing 3% skimmed milk as a supplement, the positive results of this test indicated presence of a transparent zone around the inoculum spot.

To determine the ability of *H. pylori* to produce biofilm, isolates of *H. pylori* were cultured on brain heart infusion broth with supplement sucrose and Congo red and agar added into the media.

Congo red agar (CRA) plates were incubated at 37 °C for 48 hours. The ability of *H. pylori* isolates to produce biofilm through the appearance of black dry crystalline colonies on the CRA plates indicated biofilm production, while some of the isolates that showed pink or red colonies indicated absence of biofilm.

Results

Stool examination revealed 25% of samples to have *H. pylori* growth, 52% of the patients being males and 48% females (Table 1). The growth was more prevalent in the middle age group (35–45) and was mostly associated with *H. pylori* growth (Table 2).

The results of the present study showed that the *H. pylori* isolates were gram-negative bacteria with spiral or rods that were typically arranged singly. They were also motile, positive for methyl red, catalase, urease and oxidase tests. Table 3 and Figure 1 provide details on the routine biochemical tests, colony morphology, and microscopic examination.

Table 1
Distribution of bacterial growth with gender

Gender	Number, %	Growth, %	No growth, %
Male	60 (60%)	13/25 (52%)	45/75 (60%)
Female	40 (40%)	12/25 (48%)	30/75 (40%)
Total	100 (100%)	25 (25%)	75 (75%)

Table 2
Distribution of bacterial growth with age

Ages, years	Growth, %		No growth, %	
	male	female	male	female
25–35	4/13 (30.8%)	3/12 (25.0%)	17/45 (37.8%)	14/30 (46.7%)
35–45	6/13 (46.2%)	4/12 (33.3%)	15/45 (33.3%)	9/30 (30.0%)
45–50	3/13 (23.0%)	5/12 (41.7%)	13/45 (28.9%)	7/30 (23.3%)
Total	13 (100.0%)	12 (100.0%)	45 (100.0%)	30 (100.0%)

Table 3
Identification results and some biochemical tests for *H. pylori* isolates

Test	Result
Gram stain	Gram-negative
Growth on Columbia agar media	small, convex, translucent, non-hemolytic colony
Catalase	positive
Urease	positive
Oxidase	positive
Motility	motile
Indol production	negative
Methyl red	positive
Citrate Utilization	positive
Alkaline Phosphatase	positive

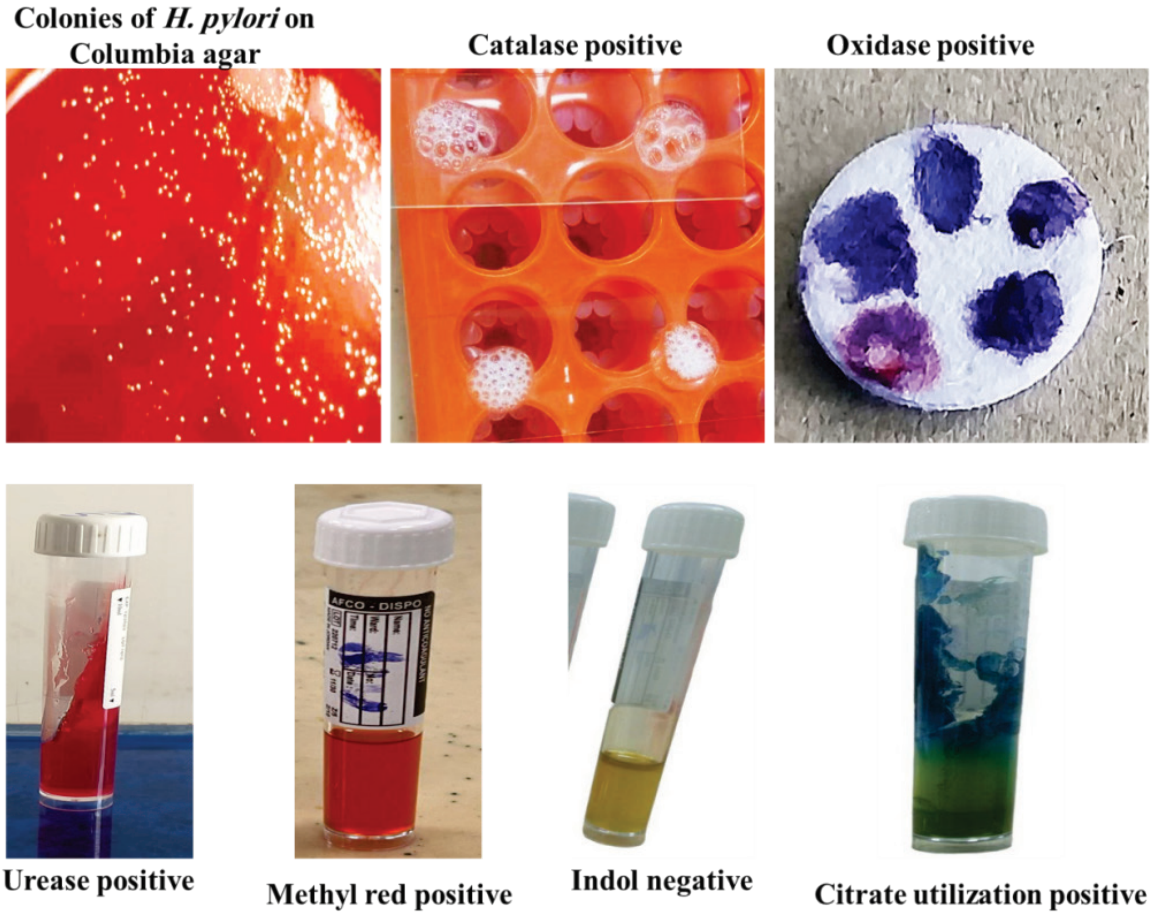

Fig. 1. Biochemical tests for *H. pylori* isolates showed positive results for catalase, oxidase, urease, methyl red, indol, and citrate utilization when cultured on Columbia Agar media

The sensitivity tests for those samples which showed *H. pylori* growth (n = 25), indicated that the resistance percentage to metronidazole (MICs ≥ 6 µg/L) was 36%, amoxicillin MICs ≤ 1 µg/mL was 28%, while resistance rates were 6/25 (24%) to tetracycline and 3/25 (12%) to clarithromycin (MICs ≤ 4 µg/mL). The MIC values for clarithromycin ranged from 0.12 to 4 µg/mL, while those for amoxicillin and tetracycline were between 0.12 and 8 µg/mL and 0.125 to > 32 for metronidazole as shown in Table 4, Figures 2 and 3.

Table 4
Distribution of antibiotics resistance among *H. pylori* isolates

Antibiotics	Sensitive	Resistance	MIC 50, µg/mL	MIC 90, µg/mL	MIC range, µg/mL
Metronidazole	16/25 (64%)	9/25 (36%)	6.0	12	0.13 – > 32
Clarithromycin	22/25 (88%)	3/25 (12%)	0.8	4	0.12–4
Amoxicillin	18/25 (72%)	7/25 (28%)	0.5	1	0.12–8
Tetracycline	19/25 (76%)	6/25 (24%)	1.0	4	0.12–8

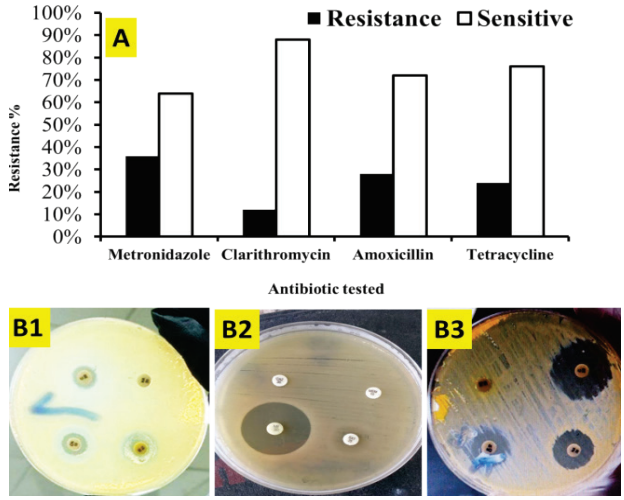

Fig. 2. Result of antibiotic sensitivity test of the tested drugs (*A*) antimicrobial susceptibility pattern of *H. pylori* isolates; (*B*) representative images of the tested drugs

Phenotypic detection of virulence factors among H. pylori isolates. The bacteria were tested for the production of virulence factors including the production of protease, lipase, and biofilm production (Table 5 and Fig. 3).

Table 5
The outcome of reactions of virulence factors

Virulence	Positive	Negative
Protease	18/25 (72%)	7/25 (28%)
Lipase	16/25 (64%)	9/25 (36%)
Biofilm	21/25 (84%)	4/25 (16%)

Discussion

The present study confirmed that one-quarter of patients visiting the gastroenterology department in the hospital were positive for *H. pylori* and most often included middle-aged patients. The isolates were resistant to tested drugs (metronidazole, clarithromycin, amoxicillin and tetracycline), confirmed by antibiotic sensitivity and virulence factors produced by the isolated bacteria. Previously, *H. pylori* isolates were susceptible to a wide range of antibiotics, but over time, many bacterial isolates have become resistant to many antibiotics on a large scale, making it difficult to obtain appropriate treatment for the bacteria. Therefore, it is very important to conduct research to determine the extent of the bacteria's sensitivity or resistance.

Earlier studies agreed with this study regarding the percentage of *H. pylori* positive sample in the general population, since Saber & Ali (2022) reported nearly one-third distribution in their tested samples and the distribution in their study was higher in the middle-aged (40–50 years) group than the younger (<40 years) and older group (>50 years). Conversely, Fabricio Guaman et al., (2018) reported that the prevalence was similar in the middle-aged group and the younger group (<40 years). This could be explained partially in terms of the higher stress exposure in the middle age group, which might reduce immunity and hence the presence of infection (Martin et al., 2001). Nonetheless, pollution, social status, lifestyle, food types, smoking, rural residency, inadequate family income should not be ruled out (Kouitcheu Mabeku et al., 2018; Kotilea et al., 2019; Bashir & Khan, 2023; Ibrahim, 2024).

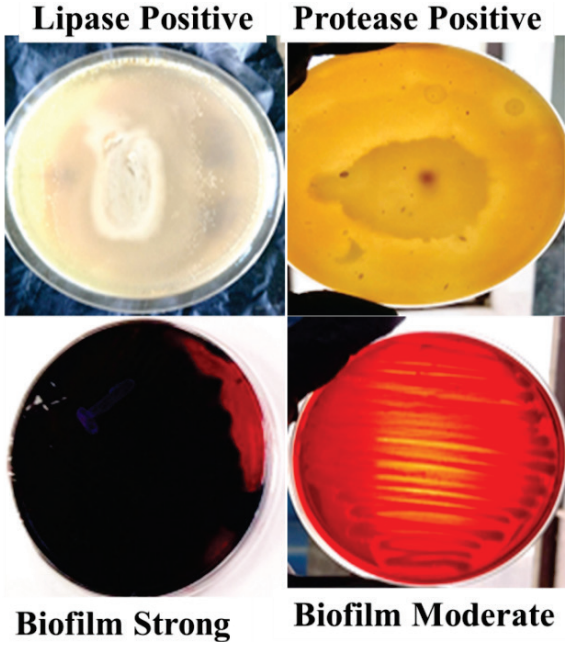

Fig. 3. Representative images for the virulence factor tests conducted on isolated *H. pylori* showing phenotypic detection of protease, lipase enzymes, and biofilm production by Congo red agar among *H. pylori*

Gender has shown no contribution to the prevalence of *H. pylori* between males and females. Similarly, Şeyda et al. (2007) reported non-significant differences between males and females. Nonetheless, the percentage of *H. pylori* distribution in females was higher than in males with prevalence increasing in females with aging.

Despite that, it has been reported that the prevalence of *H. pylori* is more prevalent in developed countries compared to developing countries (Hu et al., 2016). In this study, only one-third of positive isolates were resistant to metronidazole and this result has been reported with previous studies, for instance, in studies conducted in Morroco (Bouihat et al., 2017), Indonesia (Miftahussurur et al., 2016), Singapore (Ang et al., 2016), Spain (Alarcón et al., 2017), Italy (Di Giulio et al., 2016), Bulgaria (Boyanova et al., 2016), Poland (Karpiński et al., 2015) and Germany (Regnath et al., 2017). Nevertheless, very low resistance (1%) was reported in Iceland (Gunnarsdottir et al., 2017) and very high resistance (75–95%) was reported in Iran (Goudarzi et al., 2016), China, and the USA (Chen et al., 2017). The mechanism of resistance by metronidazole is not fully elucidated, although several reports have confirmed that *H. pylori* offered metronidazole resistance in four aspects, including increased antibiotic efflux, decreased antibiotic uptake through the bacterial wall, decreased mechanisms that are necessary for metronidazole activation, increased free radical production leading to DNA damage (Hanafi et al., 2016; Hu et al., 2016).

One of the main causes of the enhanced resistance to metronidazole that *H. pylori* isolates exhibit is genetic drift in addition to adaptation to different environmental conditions (Kim et al., 2009; Shokrzadeh et al., 2011). Other causes may be the common usage of metronidazole for the management of bacterial and parasitic infections, diarrheal illnesses, as well as gastritis, and environmental conditions in terms of incubation period, dosage of vaccines, age of bacterial colonies, and choice of medium. All of these factors cause a notable

rise in the metronidazole resistance by *H. pylori* isolates (Landstedt et al., 2017; Mollin et al., 2022).

Clarithromycin has increasingly reported as exhibiting low levels of *H. pylori* resistance, as was the case with our study which reported up to 12% resistance, which does agree with previous reports in China (Wang et al., 2019), Taiwan (Liang et al., 2020), France (Mégraud et al., 2021), Russia (Dekhnich et al., 2018), Spain (Botija et al., 2021), Chile (Parra-Sepúlveda et al., 2019), and Columbia (Álvarez et al., 2009; Bedoya-Gómez et al., 2020). Nevertheless, increased resistance has been reported in other areas of the world, including Iran (Sholeh et al., 2020), South East Asia (Savoldi et al., 2018), Australia (Schubert et al., 2022), Belgium (Miendje Deyi et al., 2011, 2019), Bulgaria (Boyanova et al., 2016), and Italy (Saracino et al., 2020).

The resistance mechanism against clarithromycin is multifactorial, including the wide spectrum use of macrolide for treatment of various diseases, such as upper and lower respiratory tract infections and sexually transmitted infections; this makes it highly susceptible to resistance.However, this could be overcome by triple therapy eradication of *H. pylori* (Bedoya-Gómez et al., 2020; Mégraud et al., 2021). Moreover, the frequent application of macrolides for the treatment of COVID-19 has been cited as a cause of macrolide resistance (Darweesh et al., 2021), a situation which is exacerbated by the frequency of transportation between countries of high macrolide resistance to low macrolide resistance (Schubert et al., 2022). At the molecular level, clarithromycin resistance was most often linked to A2142C, A2142G and A2143G mutations in the V domain of 23S rRNA, and some minor mutations outside the domain (Tshibangu-Kabamba & Yamaoka, 2021).

Helicobacter pylori has shown a high rate of amoxicillin resistance reaching up to 28%. Similarly, increased amoxicillin resistance has been reported in Iran (Sholeh et al., 2020), China (Wang et al., 2019), Vietnam (Tran et al., 2022), and Bulgaria (Boyanova et al., 2022). Conversely, no resistance was reported in Taiwan (Liang et al., 2020), Australia (Schubert et al., 2022), Belgium (Miendje Deyi et al., 2011), France (Mégraud et al., 2021), Russia (Dekhnich et al., 2018), Chile (Parra-Sepúlveda et al., 2019), and Columbia (Álvarez et al., 2009). Mutations have been reported in penicillin-binding protein-2 (PBP2) or PBP3 and mutations leading to diminished binding of the agent to penicillin-binding protein PBP1A (Tshibangu-Kabamba & Yamaoka, 2021).

Moreover, amoxicillin resistance also developed from the frequent use of amoxicillin in treatment of different infectious diseases (Tshibangu-Kabamba & Yamaoka, 2021). Amoxicillin resistance is mainly associated with mutations of the penicillin-binding protein 1A gene (Hu et al., 2016).

Helicobacter pylori has shown high rate of tetracycline resistance reaching up to 24%. Similarly, increased tetracycline resistance has been reported in Iran (Sholeh et al., 2020). Conversely, no resistance was reported in China (Wang et al., 2019), Taiwan (Liang et al., 2020), Australia (Schubert et al., 2022), Belgium (Miendje Deyi et al., 2011), Bulgaria (Boyanova et al., 2016), France (Mégraud et al., 2021), Russia (Dekhnich et al., 2018), Spain (Botija et al., 2021), Chile (Parra-Sepúlveda et al., 2019), Columbia (Álvarez et al., 2009). *Helicobacter pylori* resistance to tetracycline was associated with single, double and especially simultaneous triple point mutations within both copies (rrnA/B genes) of 16S rRNA (Seriki et al., 2018). Tetracycline resistance is mainly associated with mutations in the 16S rRNA gene (Hu et al., 2016).

Conclusion

The resistance profile of the antibiotics used in the present study demonstrated that the resistance was highest with metronidazole at 36%, while the resistance rate was lower with clarithromycin, amoxicillin, and tetracycline, showing 12%, 28%, and 24% resistance, respectively. Proper use of antibiotics should be advised by pharmacists and physicians to avoid further decline in antibiotic sensitivity.

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References

Alarcón, T., Urruzuno, P., Martínez, M. J., Domingo, D., Llorca, L., Correa, A., & López-Brea, M. (2017). Antimicrobial susceptibility of 6 antimicrobial agents in *Helicobacter pylori* clinical isolates by using EUCAST breakpoints compared with previously used breakpoints. Enfermedades infecciosas y microbiologia clinica, 35(5), 278–282.

Álvarez, A., Moncayo, J. I., Santacruz, J. J., Santacoloma, M., Corredor, L. F., & Reinosa, E. (2009). Antimicrobial susceptibility and mutations involved in clarithromycin resistance in *Helicobacter pylori* isolates from patients in the Western Central Region of Colombia. Antimicrobial Agents and Chemotherapy, 53(9), 4022–4024.

Ang, T. L., Fock, K. M., Ang, D., Kwek, A. B. E., Teo, E. K., & Dhamodaran, S. (2016). The changing profile of *Helicobacter pylori* antibiotic resistance in Singapore: A 15-year study. Helicobacter, 21(4), 261–265.

Bashir, S. K., & Khan, M. B. (2023). Overview of *Helicobacter pylori* infection, prevalence, risk factors, and its prevention. Advanced Gut and Microbiome Research, 2023, 9747027.

Bedoya-Gómez, I. J., Alvarez-Aldana, A., Moncayo-Ortiz, J. I., Guaca-González, Y. M., Santacruz-Ibarra, J. J., Arturo-Arias, B. L., Castañeda-Chávez, L. J., Leon Rodriguez, D. A., & Beltrán-Angarita, L. (2020). Surveillance of the antimicrobial resistance rates of *Helicobacter pylori* ten years later in the Western Central Region, Colombia. Digestive Diseases, 38(3), 196–203.

Botija, G., García Rodríguez, C., Recio Linares, A., Campelo Gutiérrez, C., Pérez-Fernández, E., & Barrio Merino, A. (2021). Resistencias antibióticas y tasas de erradicación en infección por *Helicobacter pylori*. Anales de Pediatría, 95(6), 431–437.

Bouihat, N., Burucoa, C., Benkirane, A., Seddik, H., Sentissi, S., Al Bouzidi, A., Elouennas, M., & Benouda, A. (2017). *Helicobacter pylori* primary antibiotic resistance in 2015 in Morocco: A phenotypic and genotypic prospective and multicenter study. Microbial Drug Resistance, 23(6), 727–732.

Boyanova, L., Gergova, G., Evstatiev, I., Spassova, Z., Kandilarov, N., Yaneva, P., Markovska, R., & Mitov, I. (2016). *Helicobacter pylori* resistance to six antibiotics by two breakpoint systems and resistance evolution in Bulgaria. Infectious Diseases, 48(1), 56–62.

Boyanova, L., Kandilarov, N., Hadzhiyski, P., Gergova, R., Gergova, G., & Markovska, R. (2022). Increase in amoxicillin resistance in *Helicobacter pylori* from Bulgarian patients over 15 years. Diagnostic Microbiology and Infectious Disease, 104(1), 115746.

Chen, D., Cunningham, S. A., Cole, N. C., Kohner, P. C., Mandrekar, J. N., & Patel, R. (2017). Phenotypic and molecular antimicrobial susceptibility of *Helicobacter pylori*. Antimicrobial Agents and Chemotherapy, 61(4), e02530-16.

Darweesh, O., Abdulrazzaq, G. M., Al-Zidan, R. N., Bebane, P., Merkhan, M., Aldabbagh, R., & AlOmari, N. (2021). Evaluation of the pharmacologic treatment of COVID-19 pandemic in Iraq. Current Pharmacology Reports, 7(4), 171–178.

Dekhnich, N., Ivanchik, N., Kozlov, R., Alimov, A., Steshits, A., Kirsov, P., & Pandav, K. (2018). Dynamics of antimicrobial resistance of *Helicobacter pylori* isolates in the Smolensk region of Russian Federation. Helicobacter, 23(6), e12545.

Di Giulio, M., Di Campli, E., Di Bartolomeo, S., Cataldi, V., Marzio, L., Grossi, L., Ciccaglione, A. F., Nostro, A., & Cellini, L. (2016). *In vitro* antimicrobial susceptibility of *Helicobacter pylori* to nine antibiotics currently used in Central Italy. Scandinavian Journal of Gastroenterology, 51(3), 263–269.

Fabricio Guaman, J., Bayas-Morejon, I. F., Arcos, V., Tigre-Leon, A., Lucio-Quintana, A., Salazar, S., Gaibor-Chavez, J., & Ramon Curay, R. (2018). Detection of *Helicobacter pylori* from human biological samples (feces) by antigenic screening and culture. Jundishapur Journal of Microbiology, 11(7), e66721.

Goudarzi, M., Heidary, M., Azad, M., Fazeli, M., & Goudarzi, H. (2016). Evaluation of antimicrobial susceptibility and integron carriage in *Helicobacter pylori* isolates from patients. Gastroenterology and Hepatology from Bed to Bench, 9(Suppl1), S47–S52.

Gunnarsdottir, A. I., Gudjonsson, H., Hardardottir, H., Jonsdottir, K. D., & Bjornsson, E. S. (2017). Antibiotic susceptibility of *Helicobacter pylori* in Iceland. Infectious Diseases, 49(9), 647–654.

Hanafi, A., Lee, W. C., Loke, M. F., Teh, X., Shaari, A., Dinarvand, M., Lehours, P., Mégraud, F., Leow, A. H. R., Vadivelu, J., & Goh, K. L. (2016). Molecular and proteomic analysis of levofloxacin and metronidazole resistant *Helicobacter pylori*. Frontiers in Microbiology, 7, 2015.

Hu, Y., Zhang, M., Lu, B., & Dai, J. (2016). Helicobacter pylori and antibiotic resistance, a continuing and intractable problem. Helicobacter, 21(5), 349–363.

Ibrahim, M. E. (2024). Epidemiology, pathogenicity, risk factors, and management of *Helicobacter pylori* infection in Saudi Arabia. Biomolecules and Biomedicine, 24(3), 440–453.

Karpiński, T. M., Andrzejewska, E., Eder, P., Linke, K., & Szkaradkiewicz, A. (2015). Evaluation of antimicrobial resistance of *Helicobacter pylori* in the last 15 years in West Poland. Acta Microbiologica et Immunologica Hungarica, 62(3), 287–293.

Kim, S. Y., Joo, Y. M., Lee, H. S., Chung, I.-S., Yoo, Y.-J., Merrell, D. S., & Cha, J.-H. (2009). Genetic analysis of *Helicobacter pylori* clinical isolates suggests resistance to metronidazole can occur without the loss of functional rdxA. The Journal of Antibiotics, 62(1), 43–50.

Kotilea, K., Bontems, P., & Touati, E. (2019). Epidemiology, diagnosis and risk factors of *Helicobacter pylori* infection. In: Kamiya, S. & Backert, S. (Eds.). Helicobacter pylori in human diseases. Springer, Cham. Pp. 17–33.

Kouitcheu Mabeku, L. B., Noundjeu Ngamga, M. L., & Leundji, H. (2018). Potential risk factors and prevalence of *Helicobacter pylori* infection among adult patients with dyspepsia symptoms in Cameroon. BMC Infectious Diseases, 18(1), 278.

Landstedt, K., Sharma, A., Johansson, F., Stålsby Lundborg, C., & Sharma, M. (2017). Antibiotic prescriptions for inpatients having non-bacterial diagnosis at medicine departments of two private sector hospitals in Madhya Pradesh, India: A cross-sectional study. BMJ Open, 7(4), e012974.

Liang, C.-M., Tai, W.-C., Hsu, P.-I., Wu, D.-C., Kuo, C.-H., Tsay, F.-W., Lee, C.-L., Chen, K.-Y., & Chuah, S.-K. (2020). Trend of changes in antibiotic resistance in *Helicobacter pylori* from 2013 to 2019: A multicentre report from Taiwan. Therapeutic Advances in Gastroenterology, 13, 1756284820976990.

Marshall, B., & Warren, J. R. (1984). Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. The Lancet, 323(8390), 1311–1315.

Martin, M., Grunendahl, M., & Martin, P. (2001). Age differences in stress, social resources, and well-being in middle and older age. The Journals of Gerontology Series B: Psychological Sciences and Social Sciences, 56(4), 214–222.

Mégraud, F., Alix, C., Charron, P., Bénéjat, L., Ducournau, A., Bessède, E., & Lehours, P. (2021). Survey of the antimicrobial resistance of *Helicobacter pylori* in France in 2018 and evolution during the previous 5 years. Helicobacter, 26(1), e12767.

Miendje Deyi, V. Y., Bontems, P., Vanderpas, J., De Koster, E., Ntounda, R., Van Den Borre, C., Cadranel, S., & Burette, A. (2011). Multicenter survey of routine determinations of resistance of *Helicobacter pylori* to antimicrobials over the last 20 years (1990 to 2009) in Belgium. Journal of Clinical Microbiology, 49(6), 2200–2209.

Miendje Deyi, V. Y., Lare, M. S., Burette, A., Ntounda, R., Elkilic, O., Cadranel, S., Bontems, P., & Hallin, M. (2019). Update of primary *Helicobacter pylori* resistance to antimicrobials in Brussels, Belgium. Diagnostic Microbiology and Infectious Disease, 95(4), 114875.

Miftahussurur, M., Syam, A. F., Nusi, I. A., Makmun, D., Waskito, L. A., Zein, L. H., Akil, F., Uwan, W. B., Simanjuntak, D., Wibawa, I. D., Waleleng, J. B., Saudale, A. M., Yusuf, F., Mustika, S., Adi, P., Maimunah, U., Maulahela, H., Rezkitha, Y. A., Subsomwong, P., Nasronudin, … Yamaoka, Y. (2016). Surveillance of *Helicobacter pylori* antibiotic susceptibility in Indonesia: Different resistance types among regions and with novel genetic mutations. PloS One, 11(12), e0166199.

Mollin, A., Katta, M., Sobel, J. D., & Akins, R. A. (2022). Association of key species of vaginal bacteria of recurrent bacterial vaginosis patients before and after oral metronidazole therapy with short- and long-term clinical outcomes. PLoS One, 17(7), e0272012.

Parra-Sepúlveda, C., Merino, J. S., Sáez-Carrillo, K., González, C., & García-Cancino, A. (2019). Antibiotic resistance surveillance of *Helicobacter pylori* at the Biobío Region (Chile) in a decade. Arquivos de Gastroenterologia, 56(4), 361–366.

Regnath, T., Raecke, O., Enninger, A., & Ignatius, R. (2017). Increasing metronidazole and rifampicin resistance of *Helicobacter pylori* isolates obtained from children and adolescents between 2002 and 2015 in southwest Germany. Helicobacter, 22(1), e12327.

Saber, F. O., & Ali, M. K. (2022). Isolation and identification of *H. pylori* among Iraq patients with chronic gastric inflammation. Journal of the Faculty of Medicine Baghdad, 64(2), 102–108.

Saracino, I. M., Fiorini, G., Zullo, A., Pavoni, M., Saccomanno, L., & Vaira, D. (2020). Trends in primary antibiotic resistance in *H. pylori* strains isolated in Italy between 2009 and 2019. Antibiotics, 9(1), 26.

Savoldi, A., Carrara, E., Graham, D. Y., Conti, M., & Tacconelli, E. (2018). Prevalence of antibiotic resistance in *Helicobacter pylori*: A systematic review and meta-analysis in World Health Organization regions. Gastroenterology, 155(5), 1372–1382.

Schubert, J. P., Warner, M. S., Rayner, C. K., Roberts-Thomson, I. C., Mangoni, A. A., Costello, S., & Bryant, R. V. (2022). Increasing *Helicobacter pylori* clarithromycin resistance in Australia over 20 years. Internal Medicine Journal, 52(9), 1554–1560.

Semino-Mora, C., Doi, S. Q., Marty, A., Simko, V., Carlstedt, I., & Dubois, A. (2003). Intracellular and interstitial expression of *Helicobacter pylori* virulence genes in gastric precancerous intestinal metaplasia and adenocarcinoma. The Journal of Infectious Diseases, 187(8), 1165–1177.

Seriki, A. T., Smith, S. I., Adeleye, A. I., & Fowora, M. A. (2018). Molecular analysis of low-level tetracycline resistance in clinical isolates of *Helicobacter pylori* among dyspeptic patients in South West Nigeria. Journal of Global Antimicrobial Resistance, 13, 143–145.

Şeyda, T., Derya, Ç., Füsun, A., & Meliha, K. (2007). The relationship of *Helicobacter pylori* positivity with age, sex, and ABO/Rhesus blood groups in patients with gastrointestinal complaints in Turkey. Helicobacter, 12(3), 244–250.

Shokrzadeh, L., Jafari, F., Dabiri, H., Baghaei, K., Zojaji, H., Alizadeh, A., Aslani, M., & Zali, M. (2011). Antibiotic susceptibility profile of *Helicobacter pylori* isolated from the dyspepsia patients in Tehran, Iran. Saudi Journal of Gastroenterology, 17(4), 261–264.

Sholeh, M., Maleki, F., Krutova, M., Bavari, S., Golmoradi, R., Sadeghifard, N., Amiriani, T., & Kouhsari, E. (2020). The increasing antimicrobial resistance of *Helicobacter pylori* in Iran: A systematic review and meta-analysis. Helicobacter, 25(5), e12730.

Tran, T. T., Nguyen, A. T., Quach, D. T., Pham, D. T.-H., Cao, N. M., Nguyen, U. T.-H., Dang, A. N.-T., Tran, M. A., Quach, L. H., Tran, K. T., Le, N. Q., Ung, V. V., Vo, M. N.-Q., Nguyen, D. T., Ngo, K. D., Tran, T. L., & Nguyen, V. T. (2022). Emergence of amoxicillin resistance and identification of novel mutations of the pbp1A gene in *Helicobacter pylori* in Vietnam. BMC Microbiology, 22(1), 41.

Tshibangu-Kabamba, E., & Yamaoka, Y. (2021). *Helicobacter pylori* infection and antibiotic resistance – From biology to clinical implications. Nature Reviews Gastroenterology and Hepatology, 18(9), 613–629.

Wang, D., Guo, Q., Yuan, Y., & Gong, Y. (2019). The antibiotic resistance of *Helicobacter pylori* to five antibiotics and influencing factors in an area of China with a high risk of gastric cancer. BMC Microbiology, 19(1), 152.