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Study of H. pylori Resistance to Antibiotics.

RESEARCH ARTICLE

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Comparative Study of *Helicobacter Pylori* Resistance to Clarithromycin and Metronidazole and Its Association with Epidemiological Factors in A Moroccan Population

Imane Essaidi[1,2], Ghizlane Bounder[1], Reda Mohamed Jouimyi[1], Hasna Boura[1], Ilhame Elyounsi[3], Fatima-Zahra Kheir[1], Hakima Benomar[4], Wafaa Badre[5], Khalid Zerouali[2], Fatima Maachi[1]*

Abstract

Objective: Knowledge of local antibiotic resistance is crucial to the adaption of the effective empirical first-line treatment for Helicobacter Pylori (*H. pylori*) infection. This study aimed to evaluate the prevalence of *H. pylori* resistance to clarithromycin and compare it with that of metronidazole, and highlight the impact of epidemiological factors and gastric lesions severity on *H. pylori* resistance. **Methods:** The susceptibility to clarithromycin of 96 isolates was determined by PCR-RFLP and the susceptibility to metronidazole of 185 isolates was determined by classic PCR. **Result:** Our results showed that the prevalence of *H. pylori* resistance to clarithromycin (14.6%) was low compared to that recorded with metronidazole (62.7%). Moreover, we remarked that 7.3% of isolates were co-resistant to both antibiotics. The assessment of epidemiological factors' impact on the resistance to studied antibiotics has revealed no association. Besides, our results had demonstrated that the metronidazole and clarithromycin resistance was not related to the severity of gastric lesions. **Conclusion:** In our population, clarithromycin seems to be an effective antibiotic as long as the resistance rate of *H. pylori* is low. In contrast to metronidazole, it appears that this antibiotic will lose its efficacy, due to the high rate of resistance among our population. Therefore, each population must conduct their epidemiologic studies separately to survey the resistance profile of strains and choose the appropriate antibiotic, in order to avoid the failure of *H. pylori* eradication and the development of severe gastric diseases.

Keywords: *Helicobacter pylori*- Resistance- Metronidazole- Clarithromycin- Eradication

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Introduction

Helicobacter Pylori (*H. pylori*) is a Gram-negative, microaerophilic, spiral bacterium that colonizes the stomach of approximately half the world's population. In Morocco, the prevalence of *H. pylori* infection exceeds 70% (Bounder et al., 2017). This bacterium represents the main risk factor for chronic gastritis, peptic ulcer disease, gastric carcinoma, and mucosa-associated lymphoid tissue (MALT) (Cancer, s. d.). The International Agency for Research on Cancer has classified *H. pylori* as a group 1 carcinogen pathogen (Cancer, s. d.).

Eradication of *H. pylori* is an important component of treatments for peptic ulcer disease and other gastrointestinal disorders(Malfertheiner et al., 2005 ; Kabir, 2009). Several studies had indicated that the eradication of *H. pylori* could reduce the incidence of gastric cancer in patients without precancerous lesions (Malfertheiner et al., 2005 ; Kabir, 2009).

Unfortunately, the resistance of *H. pylori* to antibiotics has been increased in the last decades in most parts of the world (Mohammadi et al., 2005 ; Ben Mansour et al., 2010 Bachir et al., 2018). It is well known that the increased resistance to antibiotics plays an important role in the failure of eradication treatment (Koletzko et al., 2006). The most effective treatment regimens for *H. pylori* combine a proton pump inhibitor (PPI), and two antimicrobial agents selected from amoxicillin, a 5-nitroimidazole (usually metronidazole), or clarithromycin.

However, the efficiency of standard triple therapy containing a PPI, clarithromycin, with amoxicillin or metronidazole has been declining in many countries

*[1]Laboratory of Helicobacter pylori and Gastric Pathologies, Institut Pasteur du Maroc, Casablanca 20360, Morocco. [2]Faculty of Medicine of Casablanca, IbnRochd University Hospital Center, Hassan II University, Casablanca, Morocco. [3]Faculty of Medicine, University of Tours, France. [4]Laboratory of Histo-Cytopathology, Pasteur Institute of Morocco, Casablanca 20360, Morocco. [5]Gastroenterology Department, Ibn Rochd University Hospital Center, Casablanca 20360, Morocco. *For Correspondence: fatimamaachi09@gmail.com*

because of rising antibiotic resistance. Although, there are several factors influencing effciency of an anti-*H. pylori* therapy, *H. pylori*-resistance to antibiotics remains the most relevant. According to World Health Organization, *H. pylori* ranked among the 12 most resistant bacteria in the world. While different antibiotics have been used for the treatment of *H. pylori* infection, this bacterium has evolved several mechanisms to protect itself against antimicrobial activities, in particular for clarithromycin and metronidazole(Lu et al., 1999).

Clarithromycin is a bacteriostatic antibiotic, belonging to the macrolide group and connecting to the peptidyl transferase region of the 23S ribosomal RNA (rRNA) molecule V domain. Three-point mutations (A2143G, A2142G, A2142C) in the V domain of 23S rRNA is responsible for more than 90% of adult cases for clarithromycin resistance (Mégraud, 2004).

Metronidazole is belonging to the nitroimidazole class, and it is frequently used to treat gastrointestinal infections. Metronidazole has been adopted as an antibiotic for several decades, with added antiparasitic properties that set it apart from many other antibacterial drugs, allowing it to treat a wide variety of infections (Dingsdag and Hunter, 2018 ; Hernández Ceruelos et al., 2019). The molecular mechanism of metronidazole resistance is mainly due to the inactivation of an oxygen-insensitive NADPH nitroreductase (rdxA) as a result of a deletion in the rdxA encoding gene, and may also be boosted by mutations in the frxA gene that encodes a NAD (P) H-flavin oxidoreductase (Goodwin et al., 1998 ; Debets-Ossenkopp et al., 1999).

This study aimed to evaluate the prevalence of *H. pylori* resistance to metronidazole and clarithromycin and to highlight its relationship with epidemiological factors (gender, age, living area, alcohol-tobacco consumption) and gastric lesions characteristic of carcinogenesis.

Materials and Methods

Study population and Data collection

The present study was carried out on patients with gastric diseases consulting at Ibn Rochd University Hospital Center, Casablanca (Gastroenterology and Oncology departments), aged from 18 to 86 years. Three biopsies (1 from antrum, 1 from fundus, and 1 from lesser curvature) were taken from each enrolled patient by endoscopy, from 2017 to 2020.

Clinical information about demographic characteristics of participants including age, sex, living area, smoking, and alcohol habits was collected using a structured survey. All participants were informed about their inclusion in the study and agreed to it in writing form. The study protocol has been performed in accordance with the ethical standards of Helsinki and was approved by the committee of the Pasteur Institute of Morocco.

Histopathology Test

The biopsy samples were transported in 10% formalin and embedded in paraffin. Multiple histological sections were obtained from each biopsy. Biopsy sections were then obtained and stained with hematoxylin-eosin for the detection of gastric lesions. The blades were read by a pathologist.

DNA extraction

Whole genomic DNA was extracted from gastric biopsies using a genomic DNA extraction kit (Isolate Genomic DNA Kit, Bioline) according to the manufacturer's instructions. Then it was stored at −20 °C until use.

H. pylori detection by PCR

The ure C gene (296 bp) was amplified to detect *H. pylori* infection, using the primers described by Lu et al. PCR reaction mixture was prepared with 0.5 mM dNTPs, 1.5 mM MgC2, 0.5 µM of each primer, 1 U of DNA Polymerase (MyTaq DNA Polymerase, BioLine), and 300 ng of DNA in a final volume of 20 µL. PCR thermocycling conditions were: 1 cycle at 95°C for 1 min, 35 cycles at 95°C for 15 s, 55°C for 30 s, 72°C for 30 s and a final extension cycle at 72°C for 7 min (Lu et al., 1999).

Clarithromycin Susceptibility Detection

PCR-RFLP was performed for genotyping two mutations of the 23S rRNA gene at the position A2142 C and A2143 G.

The fragment of the gene containing these mutations was amplified using specific primers (Table 1). The PCR reaction is carried out in a final volume of 25 µl included: 300 ng of genomic DNA, 0.8 µM of primers, 0.5 mM dNTPs, 1.5 mM MgC2, and 1U of Taq polymerase (MyTaq DNA Polymerase, BioLine). The amplification conditions were as follow: initial denaturation at 95°C for 1 minute, followed by 35 cycles of 95°C for 15 seconds, 60°C for 30 sec, 72°C for 30 seconds, and a final extension at 72°C for 7 minutes. The electrophoresis on a 1 % agarose gel was performed to achieve amplicons of 768 bp.

The A2142G substitution was assessed by restriction digestion with Mbo II restriction endonuclease that recognizes the GAAGA sequence and cleaves the mutated gene to 418 and 350 bp. The A2143G substitution was assessed by Eco 31I restriction endonuclease that recognizes the AATTC sequence and cleaves the mutated gene to 350, 310, and 108 bp. The cleaved product was resolved on 2% agarose gel electrophoresis (Suzuki et al., 2013).

Metronidazole Susceptibility Detection

Deletion of a 200-bp fragment from the rdxA gene is one of the molecular mechanisms of resistance to metronidazole. To determine the gene deletion, the PCR method using specific primers was performed. In the case of susceptible strains (non-mutated *H. pylori*), the amplification of this gene by PCR leads to the production of an 850-bp fragment. In the case of the defective gene (resistant-(*H. pylori*), the PCR leads to the production of a 650-bp fragment.

The rdxA gene was amplified by PCR in a total volume of 20 µL of reaction mix included: 300 ng of genomic DNA, 0.8 µM of primers, 0.5 mM dNTPs, 1.5 mM MgC2, and 1U of Taq polymerase (MyTaq DNA Polymerase, BioLine). Thermocycling conditions of PCR were as follow: initial denaturation at 95°C for 1 minute, followed

by 35 cycles of 95°C for 15 seconds, 58°C for 30 sec, 72°C for 30 seconds, and a final extension at 72°C for 7 minutes. The PCR products were analyzed by 1.5 % agarose gel electrophoresis (Debets-Ossenkopp et al., 1999).

Statistical analysis

Data entry and analysis were performed using RStudio software. The descriptive data are presented as frequencies and means ± standard deviations (SD). The differences between the groups were analyzed with Wilcox test for continuous variables, and Chi-square test or Fisher's exact test for categorical variables. The differences were considered significant at $p < 0.05$.

Results

Description of the population studied

A total of 195 gastric biopsies were obtained from patients with histopathological diagnosis. Of all patients, 59.5% (116/195) had active chronic gastritis, and 40.5% (79/195) had precancerous lesions [26.2% (51/195) had atrophic gastritis and (14.4%) (28/195) had intestinal metaplasia]. The mean age of patients was 49.9 ± 16.4 (range 18–86years). The rate of female and male was, respectively 54.4% (106/195) and 45.6% (89/195). Of 195 patients, *H. pylori* infection was detected in 185 individuals (94.9%) based on the detection of ure C gene by PCR technique.

The prevalence of clarithromycin resistance compared to metronidazole resistance

Regarding clarithromycin resistance, our study showed that 14.6 % (14 /96) of patients are infected with *H. pylori* strains resistant to clarithromycin, indicating a relatively low level of clarithromycin resistance, while 85.4 % (82/96) are infected with clarithromycin susceptible strains. Moreover, we remarked that the A2143G mutation was the commonest among our isolates while the A2142G mutation was not found.

Concerning metronidazole resistance our results showed that 62.7% (116/185) of patients are infected with *H. pylori* strains resistant to metronidazole, indicating a high level of metronidazole resistance, compared to clarithromycin resistance (14.6 %).

Seven strains (7.3%) were resistant to both antibiotics, clarithromycin, and metronidazole.

Epidemiological factors and H pylori resistance variation by gender

Analysis of *H. pylori* antibiotic resistance by gender using the data available (Table 1and 2) showed that males and females were infected in similar proportions by clarithromycin-resistant isolates, with a rate of 14% for men versus 15.4% for women. Likewise, analysis of metronidazole resistance showed similar proportions in men (62.7%) and women (63.3 %).

Variation by age

The distribution of antibiotic-resistance patterns according to age is shown in tables 1 and 2. We found that no patient was resistant to clarithromycin in the age group of [61-90]. However, in this age group, most patients were infected with strains resistant to metronidazole with a rate of 74.4%. The age group of [51-60] was the most infected by the resistant strains of clarithromycin (20.8%). However, the difference was not statistically significant between groups.

variation by the living area

Regarding the living area, antibiotics resistance was

Table 1. Prevalence of Clarithromycin Resistance and Social Factors

Factors (n)	Clarithromycin-resistance (%)	Clarithromycin-suceptibility (%)	P-value
Gender			
Men (57)	8 (14)	49 (86)	0.99
Women (39)	6 (15.4)	33 (84.6)	
Age			
18-40 (34)	4(11.8)	30 (88.2)	
41-50 (26)	5 (19.2)	21 (80.8)	0.94
51-60 (24)	5 (20.8)	19 (79..2)	
61-90 (12)	0 (00%)	12 (100)	
The living area			
Rural (25)	5 (20)	20 (80)	0.50
Urban (71)	9 (12.7)	62 (87.3)	
Tobacco			
Yes (12)	2 (14.3)	10 (85.7)	
No (84)	14 (16.7)	70 (83.3)	0.99
Alcohol			
Yes (14)	1 (7.1)	13 (92.9)	0.99
No (82)	11(13.4)	71 (86.6)	
Total 96			

Table 2. Prevalence of Metronidazole Resistance and Epidemiological Factors

Factors (n)	Metronidazole-resistance (%)	Metronidazole-suceptibility (%)	P-value
Gender			
Men (87)	54 (62.7)	33 (37.3)	0.86
Women (98)	62 (63.3)	36 (36.7)	
Age			
18-40 (54)	35 (64)	19 (36)	
41-50 (71)	19 (43.2)	25 (56.8)	0.16
51-60 (42)	30 (71.1)	12 (28.9)	
61-90 (43)	32 (74.4)	11 (25.6)	
The living area			
Rural (59)	38 (64)	21 (36)	0.86
Urban (126)	78 (60.1)	48 (39.9)	
Tobacco			
Yes (26)	17 (65.4)	9 (34.6)	
No (159)	99 (62.2)	60(37.8)	0.93
Alcohol			
Yes (16)	10 (62.3)	6 (37.7)	
No (169)	106 (62.7)	63 (37.3)	0.99
Total 185			

Table 3. The Impact of Clarithromycin and Metronidazole Resistance on the Evolution of Gastric Lesions

Histologic lesions	Clarithromycin-resistance (%)	Clarithromycin-suceptibility (%)	Metronidazole-resistance (%)	Metronidazole-suceptibility (%)
Chronic gastritis	8 (13,3)	52 (86.7)	67 (62)	41 (38)
Precancerous lesions	6 (16,7)	30 (83.3)	49 (63,6)	28 (36.4)
P-value	0.76		0.87	

more detected in the rural area compared to the urban area in the same way for clarithromycin and metronidazole respectively (60.1 % and 64% for rural area versus 12.7% and 20% % for urban area) (Tables 1-2). However, the difference was not statistically significant.

variation by the consumption of alcohol- tobacco

The study of the impact of tobacco and the consumption of alcohol on the risk of clarithromycin and metronidazole resistance showed that there was no difference between *H. pylori* resistance and the consumption of tobacco or alcohol. These results were shown in tables 2-3.

Study of the impact of resistance on gastric lesions characteristic of carcinogenesis

It is well known that *H. pylori* infection is associated with the development of precancerous lesions such as atrophic gastritis, intestinal metaplasia, and gastric cancer. For that, we tried to study the relationship between the resistance of antibiotics (clarithromycin- metronidazole), and the degree of severity of precancerous gastric lesions. Our patients were categorized into two groups according to the severity of gastric lesions related to *H. pylori* infection: chronic gastritis and precancerous lesions (atrophic gastritis and intestinal metaplasia). Clarithromycin -resistance rates in each of these two groups were respectively 13,3% (8/60) and 16,7% (6/36). The corresponding Metronidazole -resistance rates were 62% (67/108) and 63,6% (49/77) respectively. The difference was not statistically significant.

Discussion

Antibiotic susceptibilities

The high antibiotic resistance increases the risk of *H. pylori* transmission among the population and the persistence of infection that leads to gastric mucosa inflammation and increases the risk of gastric cancer (Cancer, s. d.).

The contributory factors to treatment failure are multidimensional and complex. Epidemiological factors, host genetic factors, *H. pylori* virulent factors, antibiotic-resistant *H. pylori* strains, compliance to therapy, and duration of therapy affect the treatment outcome. However, *H. pylori*-resistance to antibiotics remains the most relevant factor of *H. pylori*.

Clarithromycin is the core antibiotic of the standard triple regimen. Many studies have found that resistance to clarithromycin plays an important role in standard triple treatment failure. Recently, primary clarithromycin resistance to *H. pylori* remains a serious problem in most regions of the world (Nogueira et al., 2001 ; Datta et al., 2005 ; Hu et al., 2017 ; Bachir et al., 2018). Metronidazole is an important antibiotic in quadruple therapy. The antibiotic resistance of metronidazole is known to be as high as 50%-80% in developing or developed countries(Bachir et al., 2018).

in 2014, a Moroccan study had revealed a rate of 28.8% for clarithromycin resistance and 40.1% for metronidazole resistance (Bouihat et al., 2017). Compared to our present study, we remarked a decrease in the rate of clarithromycin resistance (14.6%) and an increase in the rate of metronidazole resistance (62.7%). These reports show that clarithromycin will have a good effect on the eradication of *H. pylori* for our population, due to the low rate of resistance to this antibiotic.

Regarding clarithromycin resistance and compared to other countries, the prevalence of resistance in our population (14.6%) seems to be similar to those found in Tunisia (14.6%) (Ben Mansour et al., 2010) and in Spain (17.1%) (Tamayo et al., 2017), lower to that detected in Iran (17%) (Mohammedi et al.,2003), in Turkish (27.6%) (Baglan PH et al.,2006) and in European countries (43.5%) (Soloca et al., 2001), and higher than that found in Congo (1.7%) (Ontsira Ngoyi et al., 2015) and Iceland (9%) (Gunnarsdottir et al., 2017). The difference in the clarithromycine resistance rate between several countries might be due to the prescription of this antibiotic.

In general, and in many countries over the world, and since clarithromycin is widely used as an antimicrobial drug to heal other infections such as respiratory infectious diseases and previous consumption of macrolides, the prevalence of clarithromycin-resistant is continuously increasing. In Europe, a positive correlation was established between long-acting macrolide consumption and *H. pylori* resistance to clarithromycin. However, in our population, the rate of clarithromycin resistance remains low because macrolides are not frequently prescribed in Morocco apart from *H. pylori* eradication. So Morocco is among the countries where it is still possible to use the standard triple therapy, which uses clarithromycin as empiric first-line treatment for this infection (Megraud et al.,2013).

Concerning the point mutations conferring resistance to clarithromycin, we found that A2143G was dominant and the A2142G was not detected in all samples. The A2143G was more prevalent in two Spanish and Tunisian studies (Alarcón et al., 2000; Bachir et al., 2018). De Francesco (2010) (De Francesco et al., 2010) reported that the A2143G was prevalent in their Italian study and concluded that this mutation reduces markedly the eradication therapy efficacy. While Hansomburana (2012) (Hansomburana et al., 2012) documented that the A2142G mutation was more frequent than the A2143G in the

Thailand population.

Regarding metronidazole resistance, our rate (62.7%) was similar to the rate found in Egypt(62.9%) (Ramzy et al., 2016), in Tunisia (56.8%) (Ben Mansour et al., 2010), in Algeria (67.5%)(Bachir et al., 2018) and in China (63.8%) (Hu et al., 2017), but was higher than that found in Europe (33.1%) (Lerang et al., 1997 ; Boyanova, 2009) and Iceland (1%) (Gunnarsdottir et al., 2017) and India (41.9%) (Bhatia et al., 2004), and lower than that found in Columbia (82%)(Alvarez et al., 2009). In a multicenter African study, despite the high heterogeneity of the studies, the overall metronidazole resistance was 75.8% which is significantly higher than that observed in Europe and South America (Jaka et al., 2018). The use of metronidazole in the treatment of other endemic diseases such as diarrheal and protozoa diseases could explain the significantly high rate of metronidazole resistance in Africa (Glupczynski et al., 2001 ; Frenck and Clemens, 2003) . Due to the increase of *H. pylori* resistance to metronidazole in our population, it appears that this antibiotic will lose its effectiveness, and it will become a significant limitation for the eradication of *H. pylori*.

Therefore, each population must conduct their own epidemiologic studies separately to survey the resistance profile and choose the appropriate antibiotic, in order to avoid the failure of *H. pylori* eradication and the development of severe gastric diseases.

Epidemiological factors and clarithromycin resistance

In this study, we found that the resistance rate to clarithromycin for males was like females. On the opposite, other studies found males are more predisposed to clarithromycin resistance than females (Boyanova et al., 2012 ; Wang et al., 2019) .

According to age, even if our result was not significant, the absence of clarithromycin resistance in elderly patients [60-90] can be explained by the non-use of macrolides in their childhood. It was noted that the prevalence of clarithromycin resistance was more frequent in patients whose age is less than 60 years. This is most likely due to the wide use of macrolides in the treatment of upper respiratory tract infections in Morocco at a young age in the last decades. Serrano (2017) found infected pediatric patients, with a high prevalence of clarithromycin resistance which is indicative of an increased prevalence of this resistance among *H. pylori* early in childhood. (Serrano et al., 2017).

The study of the impact of tobacco and the consumption of alcohol on clarithromycin resistance showed that there was no association statistically significant.

As we have already noted, *H. pylori* infection is an important etiological factor for gastric cancer, and its eradication reduces the risk of cancer development (Kabir, 2005; Malfertheiner et al., 2005; Courillon-Mallet A,2014).

The observed link between antibiotic resistance and disease severity is not well known until now. We have not found a significant difference in the frequency of clarithromycin resistance according to the severity of precancerous gastric lesions (p-value>0.05). However, Farzi (2009) found a significant difference in the frequency of clarithromycin resistance in patients with severe gastritis compared to those with chronic gastritis (Farzi et al.,2019). Exacerbation of gastric disease after ineffective treatment occurs in most of the patients, which is accompanied by the dominance of resistant strains in the stomach. In this view, the resistant strains may act like innocent bystanders, and their persistence in the gastric tissues, due to its resistance, has probably an impact on the evolution of lesions towards gastric cancer.

Epidemiological factors and metronidazole resistance

Several authors have suggested that the prior use of metronidazole for other indications, such as gynecologic infections, could account for the resistance increase (Hu et al., 2017 ; Bachir et al., 2018). This would also explain the higher prevalence of metronidazole resistance in women that has been observed in several studies (Hu et al., 2017 ; Bachir et al., 2018). Our study, however, did not show a significant difference between men and women or a more apparent increase in women (p value=0.86).

In the present study, the distribution of metronidazole resistance according to age showed that the prevalence of metronidazole resistance is high in all age groups except in the age groups of [41-50] with no statistical significance difference (p-value = 0.16) (Table 2). Also, in a Korean study, they didn't find a relationship between the prevalence of metronidazole resistance and age (Lee et al., 2013). In contrast, metronidazole resistance was significantly associated with age in an American and Japanese studies (Okuda et al., 2019; White et al., 2022).

Our results showed also that the living area, alcohol, and tobacco consumption have no impact on the resistance to metronidazole.

In this study, we investigated the association of *H. pylori* resistance to metronidazole with the severity of gastric lesions. Similar to our results (P-value=0.87) ,a Mexican study concluded that the resistance to metronidazole does not depend on the level of tissue damage(Chihu et al., 2005).

Our study has some limitations: first, although metronidazole and clarithromycin resistances were found in 62.7% and 14.6% respectively of *H. pylori*-positive cases, these percentages could be still underestimated due to the presence of other mutations which were not detected in our study. Second, the nature of the study requires a larger population size.

In conclusion, our study demonstrates that clarithromycin can be an effective antibiotic in our country, as long as the resistance rate of *H. pylori* to this antibiotic is low. In contrast to metronidazole, it appears that this antibiotic will lose its effectiveness, due to the increase of resistance rate. The epidemiological factors and the severity of gastric lesions showed no association with the resistance to metronidazole and clarithromycin. Our findings will be beneficial for the selection of an adequate *H. pylori* eradication program and avoid the development of severe gastric diseases. To increase the eradication rate to a clinically appropriate level, ongoing research on *H. pylori* resistance should be continued.

Author Contribution Statement

All authors contributed equally in this study.

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Approval

This work is part of an approved thesis.

Ethical Declaration

The study protocol has been performed in accordance with the ethical standards of Helsinki and was approved by the committee of the Pasteur Institute of Morocco.

Data Availability

The data used to support the findings of this study are included in the article.

Conflict of Interest

The authors report no conflict of interest.

References

Alarcón T, Domingo D, Prieto, N, López-Brea M (2000). Clarithromycin resistance stability in Helicobacter Pylori. Influence of the MIC and type of mutation in the 23S rRNA. Antimicrob. *Agents Chemother*, **46**, 613-6.

Alvarez A, Moncayo JI, Santacruz JJ, et al (2009). Antimicrobial susceptibility and mutations involved in clarithromycin resistance in Helicobacter Pylori isolates from patients in the western central region of Colombia. Antimicrob. *Agents Chemother*, **53**, 4022-4.

Bachir M, Allem R, Tifrit A, et al (2018). Primary antibiotic resistance and its relationship with cagA and vacA genes in Helicobacter Pylori isolates from Algerian patients. *Braz J Microbiol*, **49**, 544-1.

Baglan PH, Bozdayi G, Ozkan M, et al (2006). Clarithromycin resistance prevalence and iceA gene status in Helicobacter pylori clinical isolates in Turkish patients with duodenal ulcer and functional dyspepsia. *J Microbiol*, **44**, 409-6.

Ben Mansour, K, Burucoa C, Zribi M, et al (2010). Primary resistance to clarithromycin, metronidazole and amoxicillin of Helicobacter Pylori isolated from Tunisian patients with peptic ulcers and gastritis: A prospective multicenter study. *Ann Clin Microbiol*, **9**, 22.

Bhatia V, Ahuja V, Das B, Bal C, Sharma MP (2004). Use of imidazole-based eradication regimens for Helicobacter Pylori should be abandoned in North India regardless of in vitro antibiotic sensitivity. *J Gastroenterol Hepatol*, **19**, 619-5.

Bouihat N, Burucoa C, Benkirane, et al (2017). Helicobacter Pylori Primary Antibiotic Resistance in 2015 in Morocco: A Phenotypic and Genotypic Prospective and Multicenter Study. *Microb Drug Resist*, **23**, 727-2.

Bounder G, Boura H, Nadifiyine S, et al (2017). Epidemiology of Helicobacter Pylori Infection and Related Gastric Pathologies in Moroccan Population. *J Life Sci*, **11**.

Boyanova L (2009). Prevalence of multidrug-resistant Helicobacter Pylori in Bulgaria. *J Med Microbiol*, **58**, 930-5.

Boyanova L, Ilieva J, Gergova G, et al (2012). Numerous risk factors for Helicobacter Pylori antibiotic resistance revealed by extended anamnesis: A Bulgarian study. *J Med Microbiol*, **61**, 85-3.

Cancer I. Schistosomes, liver flukes and Helicobacter Pylori. Schistosomes, Liver Flukes and Helicobacter Pylori . IARC Working Group on the Evaluation of Carcinogenic Risks to Humans: Schistosomes.

Chihu L, Ayala G, Mohar A , et al (2005). Antimicrobial resistance and characterization of Helicobacter Pylori strains isolated from Mexican adults with clinical outcome. *J Chemother*, **17**, 270-6.

Courillon-Mallet A (2014). Follow-up of patients after Helicobacter pylori eradication. *Rev Prat*, **64**, 211-4.

Datta S, Chattopadhyay, S, Patra R, et al (2005). Most Helicobacter Pylori strains of Kolkata in India are resistant to metronidazole but susceptible to other drugs commonly used for eradication and ulcer therapy. *AP&T*, **22**, 51-7.

De Francesco V, Zullo A, Ierardi E, et al (2010). Phenotypic and genotypic Helicobacter Pylori clarithromycin resistance and therapeutic outcome: Benefits and limits. *J Antimicrob Chemother*, **65**, 327-2.

Debets-Ossenkopp YJ, Pot RG, van Westerloo DJ, et al (1999). Insertion of mini-IS605 and deletion of adjacent sequences in the nitroreductase (rdxA) gene cause metronidazole resistance in Helicobacter Pylori NCTC11637. *J Antimicrob Chemother*, **43**, 2657-2.

Dingsdag SA, Hunter N (2018). Metronidazole: An update on metabolism, structure-cytotoxicity and resistance mechanisms. *J Antimicrob Chemother*, **73**, 265-9.

Farzi N, Behzad C, Hasan Z, et al (2019). Characterization of clarithromycin heteroresistance among Helicobacterpylori strains isolated from the antrum and corpus of the stomach. *Folia Microbiol*, **64**, 143-1.

Frenck RW, Clemens J (2003). Helicobacter in the developing world. *Microbes Infect*, **5**, 705-3.

Glupczynski Y, Mégraud F, Lopez-Brea M, et al (2001). European multicentre survey of in vitro antimicrobial resistance in Helicobacter Pylori. *Eur J Clin Microbiol Infect Dis*, **20**, 820-3.

Goodwin A, Kersulyte D, Sisson G, et al (1998). Metronidazole resistance in Helicobacter Pylori is due to null mutations in a gene (rdxA) that encodes an oxygen-insensitive NADPH nitroreductase. *Mol Microbiol*, **28**, 383-3.

Gunnarsdottir AI, Gudjonsson H, Hardardottir H, Jonsdottir KD, Bjornsson ES (2017). Antibiotic susceptibility of Helicobacter Pylori in Iceland. *Infect Dis*, **49**, 647-4.

Hansomburana P, Anantapanpong S, Sirinthornpunya S, Chuengyong K, Rojborwonwittaya J (2012). Prevalence of Single Nucleotide Mutation in Clarithromycin Resistant Gene of Helicobacter Pylori: A 32-Months Prospective Study by Using Hybridization Real Time Polymerase Chain Reaction. *J Med Assoc Thai*, **95**, 8.

Hernández CA, Romero-Quezada LC, Ruvalcaba Ledezma JC, López Contreras L (2019). Therapeutic uses of metronidazole and its side effects: An update. *Eur Rev Med Pharmacal*, **23**, 397-1.

Hu Y, Zhu Y, Lu NH (2017). Primary Antibiotic Resistance of Helicobacter Pylori in China. *Dig Dis Sci*, **62**, 1146-4.

Jaka H, Rhee JA, Östlundh L, et al (2018). The magnitude of antibiotic resistance to Helicobacter Pylori in Africa and

identified mutations which confer resistance to antibiotics: Systematic review and meta-analysis. *BMC Infect Dis*, **18**, 3.
Kabir S (2009). Effect of Helicobacter Pylori eradication on incidence of gastric cancer in human and animal models: Underlying biochemical and molecular events. *Helicobacter*, **14**, 159-1.
Koletzko S, Richy, F, Bontems P, et al (2006). Prospective multicentre study on antibiotic resistance of Helicobacter Pylori strains obtained from children living in Europe. *Gut*, **55**, 1711-6.
Lee JW, Kim N, Kim JM, et al (2013). *Helicobacter*, **18**, 206–4.
Lerang F, Moum B, Haug JB, et al (1997). Highly effective twice-daily triple therapies for Helicobacter Pylori infection and peptic ulcer disease: Does in vitro metronidazole resistance have any clinical relevance?. *Am J Gastroenterol*, **92**, 248-3.
L'OMS publie une liste de bactéries contre lesquelles il est urgent d'avoir de nouveaux antibiotiques. (S. d.). https://www.who.int/fr/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed.
Lu JJ, Perng CL, Shyu RY, et al (1999). Comparison of five PCR methods for detection of Helicobacter Pylori DNA in gastric tissues. *J Clin Microbiol*, **37**, 772-4.
Malfertheiner P, Sipponen P, Naumann M, et al (2005). Helicobacter Pylori Eradication Has the Potential to Prevent Gastric Cancer: A State-of-the-Art Critique. *Am J Gastroenterol*, **100**, 2100-5.
Mégraud F (2004). H pylori antibiotic resistance: Prevalence, importance, and advances in testing. *Gut*, **53**, 1374-4.
Megraud F (2013). Helicobacter pylori resistance to antibiotics in Europe and its relationship to antibiotic consumption. *Gut*, **62**, 34–2.
Mohammedi M, Doroud D, Massarrat S, Farahvach MJ (2003). Clarithromycin resistance in Iranian Helicobacter pylori strains before introduction of clarithromycin. *Helicobater*, **8**, 79.
Mohammadi M, Doroud, D, Mohajerani, N, Massarrat S (2005). Helicobacter Pylori antibiotic resistance in Iran. *World J Gastroenterol*, **11**, 6009-3.
Nogueira C, Figueiredo C, Carneiro F, et al (2001). Helicobacter Pylori genotypes may determine gastric histopathology. *Am J Clin Pathol*, **158**, 647-4.
Okuda M, Lin Y, Wang C, Kakiuchi T, Kikuchi S (2019). Metronidazole for Helicobacter pylori eradication therapy among children and adolescents in Japan: Overcoming controversies and concerns. *Helicobacter*, **24**, 3.
Ontsira Ngoyi EN, Atipo Ibara BI, Moyen RA, et al (2015). Molecular Detection of Helicobacter Pylori and its Antimicrobial Resistance in Brazzaville, Congo. *Helicobacter*, **20**, 316.
Ramzy I, Elgarem H, Hamza I, et al (2016). Genetic mutations affecting the first line eradication therapy of helicobacter pylori -infected Egyptian patients. *Rev Inst Med Trop Sao Paulo*, **58**, 88.
Serrano CA, Leon MA, Palma C, et al (2017). Helicobacter Pylori -Clarithromycin Resistance in Symptomatic Pediatric Patients in a High Prevalence Country. *J Pediatr Gastroenterol Nutr*, **64**, 56.
Soloca N (2001).Accurate prediction of macrolide resistance in Helicobacter pylori by a PCR line probe assay for detection of mutations in the 23 S rRNA gene: multicenter validation study. *Antimicrob Agent Chemother*, **45**,1500-4.
Suzuki RB, Lopes R AB, da Câmara Lopes GA, Hung Ho T, Sperança MA (2013). Low Helicobacter Pylori primary resistance to clarithromycin in gastric biopsy specimens from dyspeptic patients of a city in the interior of São Paulo, Brazil. *BMC Gastroenterol*, **13**, 164.
Tamayo E, Montes M, Fernández-Reyes M, et al (2017). Clarithromycin resistance in Helicobacter Pylori and its molecular determinants in Northern Spain, 2013-2015. *J Glob Antimicrob Resist*, **9**, 43-6.
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 Microbiol*, **19**, 2.
White B, Winte M, DeSipio J, Phadtare S (2022). Clinical Factors Implicated in AntibioticResistance in Helicobacter pylori Patients. *Microorganisms*, **10**, 322.