Ministry of Higher Education and Scientific Research

University of Babylon
College of Pharmacy

Immunological Detection and Antibiotic Resistance in *H.pylori* infection

A project submitted to the council of the college of pharmacy in partial fulfilment of requirement for the degree of bachelors of pharmacy B.P.S

By

Yasser Mustafa Blasim

Abdallh Samer Mahmoud

Mustafa Ahmed Marza

Supervised by:

Lec.Dr. Halah Dawood Salman

1445-1446 **2024-2025**

بسم الله الرحمن الرحيم

{وَلَمَّا بَلَغَ أَشُدَّهُ وَاسْتَوَى آتَيْنَاهُ حُكْمًا وَعِلْمًا وَكَذَلِكَ نَجْزِي الْمُحْسِنِينَ}

سورة القصص -(الآية١٤)

صدق الله العظيم

الاهداء:

اليوم وعلى مشارف اخر خطوة لأتمام مسيرتنا الاكاديمية الجامعية من جامعة بابل كلية الصيدلة بدرجة البكالوريوس في تخصص الصيدلة.

نهدي هذا البحث بدايةً بشكر الله عزوجل والحمدلله ما تم جهد ولا ختم سعي الا بفضله وما تخطى العبد من عقبات وصعوبات الا بتوفيقه ومعونته.

إلى الاساتذة الافاضل والى الاساتذة المشرفين على البحث لقد كان لي الشرف الكبير لأن اكون طالباً تحت رعايتكم الفاضلة وان استفيد من خبرتكم القيمة ومعرفتكم الواسعة لقد كنتم دائماً متفانين في تقديم الدعم والتوجيه.

ايضاً أن اعبر عن شكري العميق لمن اوصانا الله بهما براً واحساناً ولمن حملتنا تسعة اشهر وهن على وهن وسهرت ليالينا تدعي الله بتوفيقه راجيةً منه سداد طريقنا أتقدم بالشكر الى امي الغالية-الي العزيز والداي الحبيبين على دعمهما اللامتناهي وتشجيعهما المستمر خلال هذه الرحلة الاكاديمية لقد كانوا دائماً بجانبي يقدمون لي الدعم الذي لا يقدر بثمن ويحفرونه على تحقيق اهدافي.

في الختام اشكر كل من قام بتقديم الدعم والمساندة سواء كان ذلك من خلال توجيهي في عملية البحث او بتقديم المشورة والتشجيع خلال اللحظات الصعبة اتمنى ان تصل هذه الرسالة اليكم كما ينبغي وانت تعبر عن مدى امتناني وتقديري لكم واخر دعوانا ان الحمدلله رب العالمين.

Abstract:

Helicobacter pylori are Gram-negative, flagellated, spiral bacteria that commonly infect humans, with an estimated 4.4 billion individuals infected worldwide, *H. pylori* infection causes gastritis, peptic ulcer disease, mucosa- associated lymphoid tissue lymphoma (MALT) and gastric cancer. *H.pylori* eradication treatments are widely performed to reduce and healing of gastric mucosal inflammation, promote ulcer healing and minimize the incidence of gastric cancer. **Methods:** In this research, thirty samples collected from patients (male and female) suffering from *H.pylori* infections attending Internal medicine ward in Marjan teaching hospital in Hillah, serum collected from all patients used to confirm diagnosis by detection bacteria through immunological kits (Ab detection) specific for *H.pylori*, the diagnosis, identification and antibiotic sensitivity tests were performed according to standard guidelines . **Results:** the current study revealed incidence of *H.pylori* infections in Hilla city of Babylon province through period of five months, even few cases of *H.pylori* infections noticed through this period, but the complication of infection with this bacteria may lead to serious squeal such as cancer, moreover, the results indicated that some patients need to repeat treatment regimen of *H.pylori* infections and need to new therapy of *H.pylori* infections. **Conclusion:** recurrence of *H.pylori* infections and antibiotic resistance of these bacteria that act as demand for repeating or renew therapy regimen may be indicated for increased the prevalent world problem of antibiotic resistance.

1.1 Introduction to *Helicobacter Pylori*

Helicobacter pylori has been recognized as a major pathogen of humankind for nearly four decades. However, despite the impact of treatment of infected individuals and the reduced transmission of infection in communities in which socioeconomic living standards have improved, it continues to be the most common human bacterial pathogen, infecting perhaps half of the world's population [1]. As a result, it is still a major cause of morbidity and mortality worldwide. *H. pylori* infection invariably causes active chronic gastritis. In most people, this may be clinically silent throughout life, but in a substantial minority it causes gastroduodenal diseases, most importantly peptic ulcer disease, noncardia gastric cancer, and gastric mucosa associated lymphoid tissue (MALT) lymphoma. It also increases the risk of gastroduodenal ulceration and bleeding in patients who are taking nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and is responsible for symptoms in a subset of patients with functional dyspepsia. *H. pylori* has been studied intensively. A literature search reveals more than 45,000 publications [2]. A great deal has been learned about the epidemiology of infection, biology, genetics, pathophysiology, disease expression, diagnosis, and treatment. However, major gaps in our knowledge remain. The precise mode of transmission of infection remains unclear, despite many epidemiological studies that identify risk factors for infection. The determinants of disease expression are still incompletely understood, including many aspects of the host– pathogen interaction. The pathophysiology of this interaction is complex and has been reviewed in detail elsewhere [3].

1.2 Natural History

Natural history of infection *H. pylori* infection usually persists for life, unless it is treated with antibiotics or auto eradication occurs when long-standing infection causes widespread gastric mucosal atrophy and metaplasia with achlorhydria. Transient infection may occur in some infants. Reinfection after treatment in adults is uncommon in both higher-prevalence and lower prevalence regions. Reinfection may be confused with recrudescence, when infection is suppressed transiently, below the threshold of detection by tests, but has not been eradicated by antibiotics. There are variations in the virulence of different *H. pylori* strains globally. The interplay between host and environmental factors may result in differences in the expression of disease.[2]

1.3 Transmission

Transmission of infection Although there are well-described risk factors for infection, and plausible hypotheses, the precise mode of transmission has not been definitively established. Most infection appears to occur in early childhood, with a minority of cases developing in adults. There is strong evidence from epidemiology and genetic studies of person-to-person transmission particularly within families. Mothers appear to be particularly important in transmission to their young children. Ingestion of the organism seems most plausible via the gastro–oral or oral–oral route. Fecal–oral transmission appears less likely, at least in developed countries. Whether transmission occurs via water, food, household pets, or flies is still a matter of speculation[1].

1.4 Epidemiology

Epidemiology Although half of the world's population are thought to be infected with H. pylori, there is widespread variation in the prevalence of infection, between and within countries (Fig. 1).

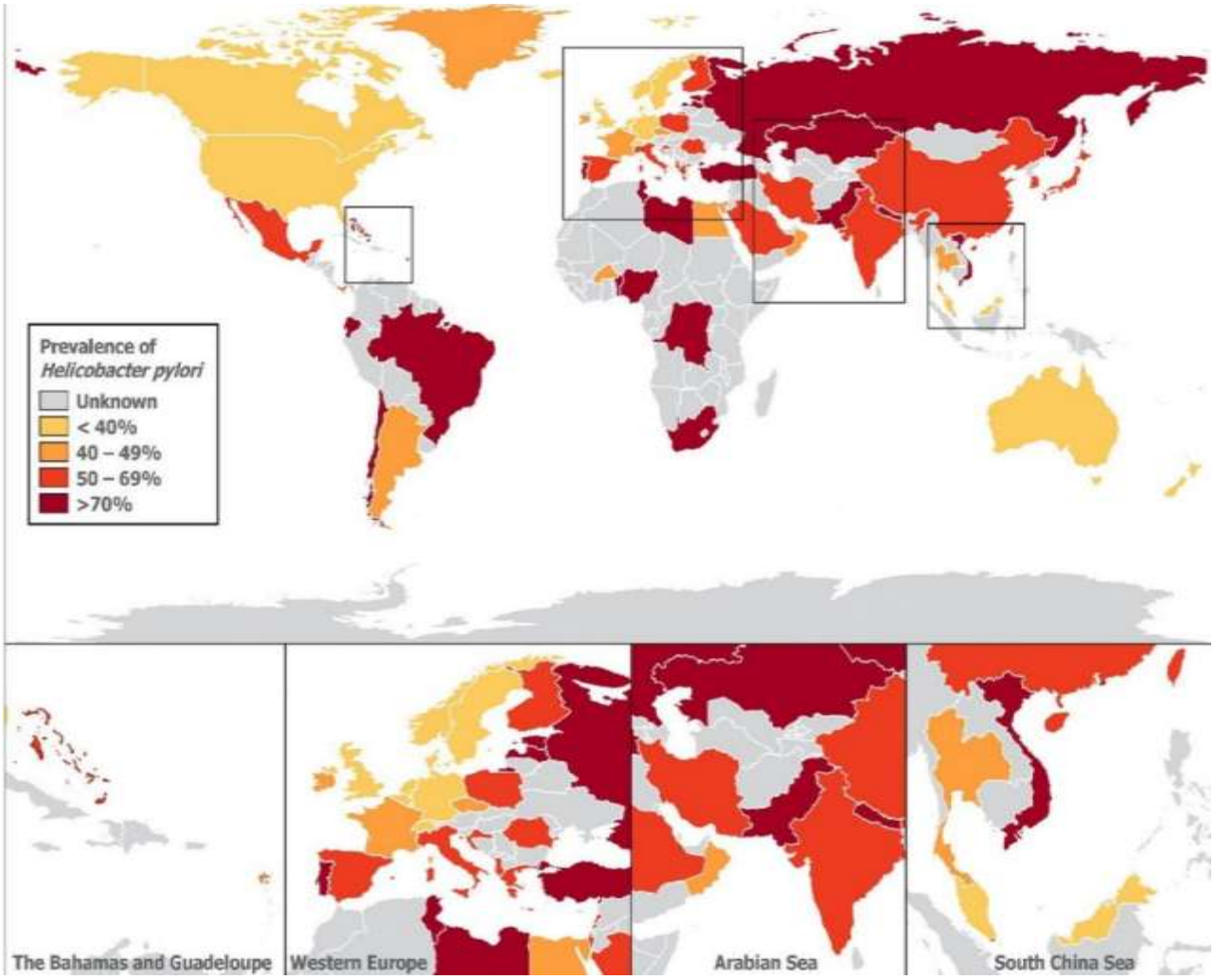

Fig. 1 Global prevalence of *H. pylori*. from Hooi *et al.* 2017 [1]

In addition, the prevalence may vary within a single city and also between subgroups within a population (Fig. 2) [4]. For example, there may be wide variations in the prevalence between more affluent urban populations and rural populations. [4] .

The quality of prevalence data varies. Many studies are not true prevalence studies, but rather audits of clinical subsets. Other studies may not represent a valid cross-section of the population. Moreover, there is significant variability in the quality of reports. In some regions, diagnostic methods may be less reliable, while some countries are poorly represented as they lack any reliable data at all. For all these reasons, a single figure cannot be taken to summarize and represent the prevalence of infection in an entire

country and must be applied with caution. For example, a prevalence study from one city in one region of a populous, multiethnic country with wide variation in socioeconomic standards is unlikely to represent the true prevalence across the entire country and cannot reflect high-risk and low-risk subsets. However, countries and regions can usually be characterized as high-prevalence, mid prevalence, and low-prevalence locations [1].

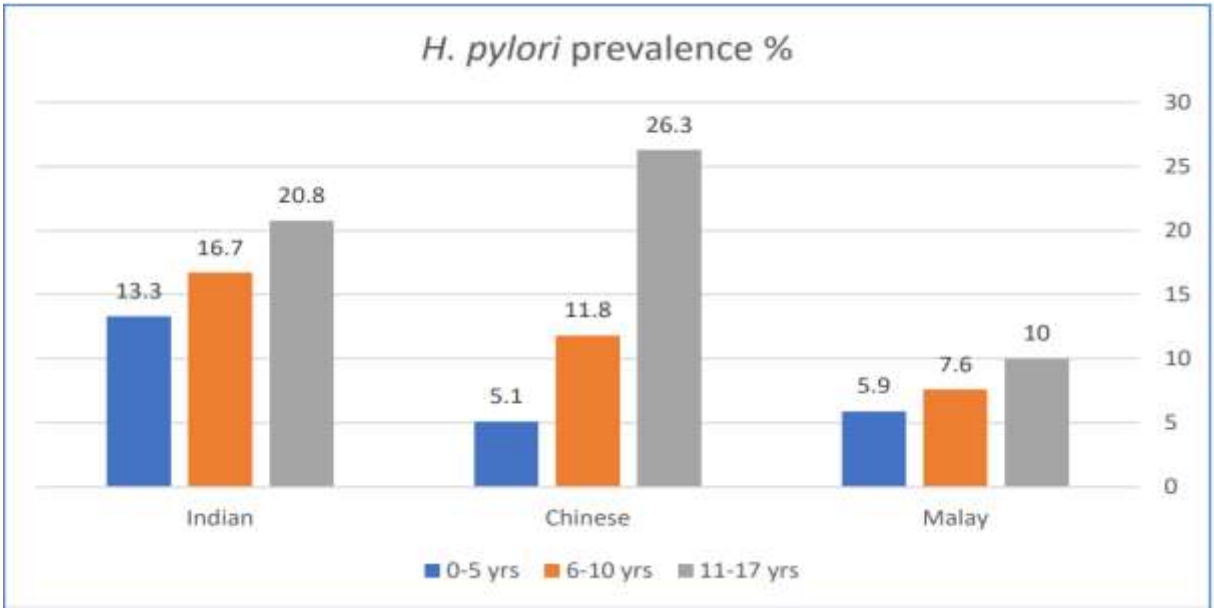

Fig. 2 Prevalence of *H. pylori* among children and young adults in Kuala Lumpur, Malaysia. From Goh [4]

1.5 Virulence factors of H pylori

The severity of H. pylori-related diseases is associated with numerous virulence factors; a particular genotype of the *H. pylori* strain plays a crucial role. Furthermore, what is of the highest importance is an interplay between the host, gastric microenvironment, as well as bacterial virulence factors. H. pylori virulence factors are not only involved in the induction of inflammatory responses, but they also control and regulate those responses, maintaining chronic inflammation. H. pylori virulence factors enable the colonization and survival of the bacterium within the gastric mucosa, leading to further immune escape and ultimately, the induction of premalignant alterations. H. pylori exhibits an expanded complex of mechanisms that alters host cellular responses and signaling pathways (Table 1).[5]

Virulence Factor	**Function**
Urease	Protects from the gastric acidity Facilitates bacterial colonization Stimulates bacterial nutrition Generates the proton motive force Modulates the host immune responses (facilitated apoptosis, chemotaxis of neutrophils and monocytes, altered opsonization, enhanced release of the pro-inflammatory cytokines) Stimulates platelet activation Stimulates angiogenesis
Flagellum	Enhances bacterial motility Stimulates chemotaxis Takes part in the biofilm formation Facilitates inflammation and immune evasion
Cytotoxin-associated gene A	Stimulates inflammatory responses Induces the release of IL-8 and IL-12 Enhances bacterial motility Activates RUNX3, ASPP2, CDX1, and fibroblasts Induces EMT Stimulates host cell growth and proliferation Reduces the activity of PDCD4, GSK-3, microRNA-134, Afadin protein, heat shock proteins Stimulates the induction of cancer stem cell-like properties
Vacuolating cytotoxin A	Involved in the formation of pores Promotes the autophagy pathways Forms the intracellular vacuoles and impaired autophagosomes Induces apoptosis and necrosis Inhibits the activity and proliferation of T and B cells Inhibits the IFN-β signaling inducing macrophage apoptosis Induces the release of IL-8 Differentiation of the regulatory T cells into effector T cells Prevents cellular elongation by inhibiting the Erk1/2 kinase pathways

Catalase	Induces mutagenesis Facilitates inflammation Protects *H. pylori* from complement-mediated killing Maintains bacterial survival at the cell surface of the phagocytes and in the macrophage phagosomes Protects *H. pylori* from phagocytosis
Superoxidase dismutase	Facilitates bacterial colonization Protects from ROS Inhibits the production of pro-inflammatory cytokines Stimulates the activation of the macrophages
Lewis antigens	Protects *H. pylori* from the host defense mechanisms Enhances bacterial survival Enhances the adhesive properties and further internalization

[6]

1.6 Diagnosis Of *Helicobacter Pylori* Infection

The decision on whether or not to treat *H. pylori* must be an active one that takes into account the individual patient's circumstances and risks. The decision to test for *H. pylori* should only be made with therapeutic intent. Evidence-based indications for testing for and treating *H. pylori* are summarized blow [7]. The applicability of each indication in different regions will depend on the prevalence of infection and disease, resources, competing needs, and individual patient factors. Peptic ulcer disease is the prime indication in most of the world. The clinical and health-economic benefits of short-term curative therapy for a common, chronic, important disease have been amply demonstrated over many years [8. In resource-poor regions, this indication for therapy should be prioritized. Indications for treatment of *H. pylori* infection :

• Past or present duodenal and/or gastric ulcer, with or without complications

• Gastric mucosa-associated lymphoid tissue (MALT) lymphoma

• Gastric mucosal atrophy and/or intestinal metaplasia

- Following resection of gastric cancer
- Patients who are first-degree relatives of patients with gastric cancer
- Patients' wishes (after full consultation with their physician)
- Functional dyspepsia
- To reduce the risk of peptic ulcer and upper gastrointestinal bleeding in nonsteroidal anti-inflammatory drug-naive users
- Before starting long-term aspirin therapy for patients at high risk for ulcers and ulcer- related complications
- Patients receiving long-term low-dose aspirin therapy who have a history of upper gastrointestinal bleeding and perforation
- Patients with gastroesophageal reflux disease who require long-term proton-pump inhibitors
- As a strategy for gastric cancer prevention in communities with a high incidence. [7]

1.6.1 Endoscopic Diagnostic Tests

Diagnostic tests for *H. pylori* infection may be invasive (endoscopic) or noninvasive (non endoscopic). Biopsies taken at endoscopy are most commonly for histological analysis and urease testing. Biopsies for culture are less often used for diagnosis, unless antimicrobial resistance testing is available and is needed to aid individual clinical decision making or determine population resistance rates. A combination of two testing modalities taken from two topographic locations in the stomach is generally most effective for diagnosis. In practice, this usually means biopsies taken from the antrum and body of the stomach for histology and from the antrum for a urease test. More structured biopsy protocols may be used when there is an additional need for histological surveillance, as in the Operative Link on Gastritis Assessment (OLGA) and Operative Link on Gastritis/Intestinal-Metaplasia Assessment (OLGIM) protocols [9]. Histology is

usually costly and very operator dependent, and accuracy cannot be assumed except in comparison with other previous testing modalities.

Culturing *H. pylori* from biopsies requires specific transport conditions, laboratory skills, and equipment. Culture success rates may reach 90% in expert centers, but are often lower than that in less expert centers. Sub culturing for antimicrobial testing may also not always be successful in less expert laboratories, so that results may not always be obtained when required. There are now commercially available real-time polymerase chain reaction (PCR) tests that allow the detection of *H. pylori* with high levels of sensitivity and specificity, and also of mutations that cause clarithromycin resistance [10]. These tests do not require strict preanalytic conditions and they can be performed in a few hours. The validation and implementation of these rapid, inexpensive kit-based point-of-care antimicrobial resistance tests promises to be a major advance in management. The availability of such tests in regions of high resistance may greatly aid the choice of therapy for individual patients, while also facilitating surveys of population prevalence[11].

1.6.2 Noninvasive Diagnostic Tests

When endoscopy is not required or not available, noninvasive tests may be used. Urea breath tests (UBTs) are very useful and have higher diagnostic accuracy than other noninvasive tests for identifying *H. pylori* (in patients without a history of gastrectomy). Somewhat surprisingly, these are not widely available in many countries in which *H. pylori* and peptic ulcer disease are most common. The reasons for this are complex, and may include a lack of expertise or resources to set up and operate breath analysis laboratories, the relatively high cost of commercial kit tests, or overreliance on either empirical therapy or endoscopy. In many cases, valid anxiety about gastric cancer is a

major driver of the use of endoscopy (although once they become symptomatic, gastric cancers are rarely curable).

Stool antigen testing is another option. These tests appear to be almost as accurate as UBTs, but patients and health-care and laboratory workers often have a lower preference for stool-based tests. Cost is an issue in some locations. Stool-based rapid PCR tests are also available [12]. Although these tests face the same acceptance barriers, as well as requiring laboratory equipment and skills, they have the potential to provide rapid diagnosis and antimicrobial resistance testing in a single noninvasive test.

Serological antibody tests are commonly available. Although they are useful as seroepidemiological surveys, these tests often lack the sensitivity and specificity required for decision-making in individual patients and are generally not very helpful. They need to be validated for specific locations, and the issue of false results due to cross-reactivity has rarely been addressed. In a community with moderate *H. pylori* prevalence, the accuracy of these tests may not exceed 50%.[13]

1.7 Treatment Of *Helicobacter Pylori* Infection

A vast number of studies have addressed therapy issues, and numerous expert guidelines recommending choices of therapy are available. However, much of the literature and advice derives from well-resourced countries, with relatively little coming from the poorly-resourced countries that bear the major burden of diseases caused by H. pylori. Principles for antibiotic therapy that apply universally have been established. However, there are key issues that must be addressed locally in order to determine the best local practice, as antimicrobial resistance patterns and therefore eradication rates vary regionally [14] and other local issues such as the cost and availability of drugs influence the choice of therapy.

1.7.1 Choice Of First-Line Eradication Therapy

Application of these principles of therapy will ensure the best outcomes possible. In well resourced regions, treatment may be based on high-quality trials and audit and culture data; in resource-poor regions, reliance on a knowledge of community or personal antibiotic usage and any local audit of outcomes will influence the use of therapies recommended in guidelines from elsewhere [15].

1.7.2 PPI, Amoxicillin, Clarithromycin Triple Therapy

In many parts of the world, triple therapy, comprising a proton-pump inhibitor (PPI) with amoxicillin and clarithromycin (PPI-AC), is still the most commonly used first-line therapy. This combination was the first very widely recommended therapy and superseded less effective triple therapies. It has been very well evaluated over the years. The major determinant of eradication success with this combination is pretreatment clarithromycin resistance (CR) [16] . The prevalence of antibiotic resistance, particularly CR, varies widely around the world. Where clarithromycin has been and is used commonly as monotherapy for other infections, the level of CR is often high and increasing. There are views that this therapy should be abandoned in areas where the primary CR rates are known to be 15–20% or greater, because of the impact this has on eradication rates. A somewhat arbitrary minimum eradication rate of 80% on an intention-to-treat basis is often quoted as a benchmark for an acceptable therapy. This is a common eradication rate for PPI-AC in real-world studies in areas where CR rates are moderate or low (i.e., below 15–20%). Unacceptably lower eradication results may occur in countries in which the prevalence of CR is higher.[17]

1.7.3 Bismuth-Based Quadruple Therapies

The other core choice for first-line therapy, especially in regions with high primary CR, is still bismuth-based quadruple therapy. The best-studied regimen involves a PPI, bismuth, tetracycline, and metronidazole (PPI-BTM). This treatment has stood the test of time, since it leads to reliable and acceptable eradication rates irrespective of primary metronidazole resistance (MR), as the addition of a PPI to BTM appears to overcome MR. Good results have been achieved with 7-day therapy, although there are proponents of longer (10–14-day) treatments. The major drawbacks of this therapy are the clumsy dosage regimen (as it is usually dosed four times daily) and common but usually mild adverse effects, which may impair adherence. Reduced access to bismuth and tetracycline may limit the use of this treatment in some places. However, when these drugs are not readily available or not registered, it is often feasible to import generic drugs at low cost, with the permission of the relevant authorities. A quadruple therapy substituting amoxicillin for tetracycline (PPI-BAM) has long been reported and is less used, but may provide acceptable outcomes. More recently, converting standard PPI-AC triple therapy to a quadruple therapy by adding bismuth (B+PPI-AC) has been reported, with favorable results in some locations [18]. The value of this in overcoming CR has yet to be fully determined, but it merits detailed evaluation.

1.7.4 Nonbismuth Quadruple Therapies

There are advocates of non-bismuth quadruple therapies—usually meaning the addition of metronidazole to PPI-AC triple therapy (PPI-ACM). This may increase eradication rates if MR rates are low or moderate, but is unlikely to be very helpful in the many regions of the world where primary MR and/or CR are high. Moreover, patients in whom the treatment fails will often be found to have dual resistance. This type of concomitant

therapy has been studied in well-resourced countries, but rarely in poorly resourced countries. Sequential or hybrid regimens are less well studied, appear not to offer superior eradication, are clumsy to prescribe, and pose particular challenges with adherence. As a result, they are not recommended. Where metronidazole sensitivity is known from testing in a patient, PPI-AM may be used as a first-line treatment with reasonable outcomes. It is also suitable in locations where MR is known to be low in the population.[18]

1.8 Overview on *Helicobacter Pylori* Resistance to Antibiotics

Almost 20 years after the establishment of the current clarithromycin-based triple therapy for the eradication of *Helicobacter pylori* [19], its efficacy is seriously challenged in many parts of the world. The aim of this first-line therapy to obtain the highest possible eradication rate, at least 80% or more, is no longer being met leading to a large amount of retreatment.

Among the possible causes of failure, which are becoming more important every year, is the decrease in the number of peptic ulcer disease treated given that the eradication rate is always higher in peptic ulcer disease than in nonulcer dyspepsia, and even more, antibiotic resistance to clarithromycin [20]. The same is also true for levofloxacin resistance; levofloxacin was proposed a decade ago as an alternative to clarithromycin. There are different ways to circumvent the problem of resistance, the most interesting of which is the use of a combination of drugs for which resistance does not appear to be a problem. Bismuth-based quadruple therapy is an attractive alternative treatment, especially in its most recent galenic formulation, bismuth subcitrate potassium, metronidazole, and tetracycline. The current situation in terms of *H. pylori* resistance to

antibiotics, alternative treatments and the relevance of BMT three-in-one capsule in clinical practice are presented, 10 years after a previous review on the topic [21].

1.9 Increased Resistance of *Helicobacter Pylori* to Clarithromycin And Levofloxacin

Antimicrobial resistance in *H. pylori* is the consequence of mutations. In the case of clarithromycin there are essentially three point mutations, which can occur at the two nucleotide positions 2142 (A2142G and A2142C) and 2143 (A2143G) in the peptidyl transferase loop of the *23S* rRNA gene; these mutations result in a conformational change leading to a decrease in binding of the drug. These mutations occur by chance, do not have an impact on bacterial fitness and can therefore remain for many generations. Furthermore, all macrolides are similarly affected by these mutations resulting in class-wide resistance [22]. Usually a low proportion of these mutants is present in the *H. pylori* population and often remains undetected by current methods. However, when a macrolide is prescribed for any infection, selection for these resistant mutants occurs and these resistant strains become the majority of the bacterial population.

The only efficient remaining drug is the second antibiotic, amoxicillin (or metronidazole), when clarithromycin-based triple therapy is prescribed to patients carrying such resistant strains. The result is a treatment regimen that is essentially an antibiotic monotherapy with limited efficacy.

The same is true for levofloxacin and all fluoroquinolones, except that the mutations occur at different locations, that is, the DNA gyrase, and are more numerous than in macrolides. The presence of these mutations prevents the inhibition of chromosome replication of the bacterium normally observed in the presence of the drug [22].

Antimicrobial resistance due to point mutations is known to increase slowly but steadily in the case of *H. pylori* resistance to clarithromycin and levofloxacin. Limited resistance to clarithromycin was present when the clarithromycin-based triple therapy was initially established in the 1990s and it has increased steadily over the last 20 years. In most European countries, as well as the rest of the world, the prevalence of clarithromycin resistance has reached 20% or more [23], and is responsible for a large number of treatment failures. Ten years later, the situation has repeated itself with regard to levofloxacin resistance, and several countries have now reached a resistance prevalence that no longer allows for levofloxacin's empiric use.

Resistance to clarithromycin and levofloxacin is mostly due to the use of these drugs for infectious diseases other than *H. pylori* infection. This explains why those countries in northern Europe, which have a strict policy for antibiotic use, still have a low prevalence of resistance. [24]

The impact of clarithromycin resistance on eradication rates was proven in clinical trials where antimicrobial susceptibility testing was performed. For example, in a meta-analysis performed by Fischbach and colleagues, the success of the triple therapy decreased by 66.2% (95% confidence interval [CI] 58.2–74.2), when the *H. pylori* strain was resistant *versus* susceptible, and while fewer studies are available, the same trend has been observed with levofloxacin [25].

1.10 Adapting Current Treatments In Areas Of High Antibiotic Resistance

1.10.1 Tailored Treatment

A logical approach is to test the antimicrobial susceptibility of *H. pylori*. Even if the prevalence of clarithromycin resistance is 25–30%, the majority of patients could benefit from the standard triple therapy while a significant number receive alternative treatments. The standard methods using culture and antimicrobial susceptibility testing (e.g. Etest, AB bioMerieux, Solna, Sweden) take several days. They can now be replaced by rapid molecular methods that detect both *H. pylori* and its resistance to macrolides. They include a standard polymerase chain reaction (PCR) [26], a real-time PCR (Engenetix, Vienna, Austria), or a fluorescence *in situ* hybridization . These methods do not require specific transport conditions, are easy to perform, and are reliable and fast. A molecular approach is also possible to test for levofloxacin resistance, which uses a commercially available multiplex PCR followed by strip hybridization [27].

There are shortcomings in using this approach however, because a number of treatments occur after a noninvasive test, the *H. pylori* antimicrobial susceptibility cannot be known and traditionally, gastroenterologists do not automatically request culture and susceptibility testing for *H. pylori*, while this approach (Culture and Susceptibility testing) is widespread in the general infectious disease specialty practice. We can expect that the availability of molecular methods will be an incentive for development in this domain.

1.10.2 'Sequential' Treatment

The impact of resistance can be minimized when drugs are prescribed sequentially instead of concomitantly. Zullo and colleagues proposed using a proton-pump inhibitor (PPI) and amoxicillin for 5 days followed by a PPI with clarithromycin and metronidazole for the next 5 days [28]; this regimen turned out to be effective for *H. pylori* strains resistant to clarithromycin. When susceptibility testing was performed it clearly showed that when clarithromycin-resistant strains were present, the outcome was better with sequential therapy (72%) than with standard therapy (33%) [29].

The hypothesis explaining the better results from administering the drugs sequentially is that during the first phase of the treatment, amoxicillin probably decreases the bacterial load, eliminating most, if not all, of the clarithromycin-resistant mutants, which represent a small proportion of the initial *H. pylori* population. The second phase of the treatment then allows the eradication of the remaining bacteria in the absence of clarithromycin-resistant mutants.[29]

A number of variations in this sequential treatment have been tested, with modifications to the length of treatment (4–7 days for each phase) and/or the drugs used. A levofloxacin sequential treatment has also been tested with variable success; 82.5% eradication in Spain and 96% in southern Italy [30].

The sequential treatment is considered to be a quadruple therapy by some and it was proposed to give the drugs concomitantly instead of sequentially. Hence, the 'concomitant' therapy originally proposed by Treiber and colleagues has been recently re-evaluated [31]. Although the results are good, the ecological impact of this regimen can be expected to be quite negative with the selection of multi resistant strains despite a

heavy antibiotic load received by the patients and, in a number of cases, the use of clarithromycin is not appropriate since it has no beneficial impact on *H. pylori*-resistant strains. [31]

1.11 Bismuth-Based Quadruple Therapy

The main concern of this alternative treatment is to avoid the major but problematic antibiotics, that is, clarithromycin and levofloxacin.

Soon after the discovery of *H. pylori*, Marshall and colleagues' review of past literature showed that some antimicrobial compounds (e.g. bismuth salts and metronidazole), had been used to treat peptic ulcer disease in the past with some success. He then used this combination in a double-blind trial *versus* cimetidine, observing the eradication of *H. pylori* in most patients treated with the combination and a low relapse rate of duodenal ulcer after 1 year [32].

Treatments are changing for several reasons. First, in the context of increased resistance to antibiotics, as seen previously, the quadruple therapy has the advantage of using the following compounds.

1. Bismuth salts is a compound with a short-term effect that acts topically; the mechanism of action is not known but appears to be more like an antiseptic than an antibiotic, and no resistance has been described;
2. Tetracycline is an antibiotic for which resistance is rarely encountered. The reason is that to reach a high level resistance, three adjacent point mutations are required. The change in the nucleotide triplet (AGA-926 to 928→TTC) has been associated with this resistance probably because of a lack of binding to the h1 loop, which is the

binding site of tetracycline on the 30S subunit of the ribosome. The two 16S rRNA copies are both involved. Simple or dual mutations at these positions lead to intermediary minimum inhibitory concentrations. The probability of finding these mutations on the same organism is extremely low and explains why this resistance does not occur. Resistance to tetracycline involving an efflux mechanism has also been described.

3. For the third antimicrobial compound, metronidazole, resistance *in vitro* exists at a high prevalence in most countries around the world, but the clinical impact of this resistance is limited and it can be overcome by increasing the dose and duration of treatment. The meta-analysis of Fischbach and Evans reports a 14% decrease in treatment success when this resistance is present *in vitro* [24,33,34]. Secondly, a specific galenic formulation of BMT three-in-one capsule has been developed, which allows standardization of the treatment with a possible positive impact on compliance and hence on eradication rates.[34]

2. Materials and Methods:

2.1-Collection of Samples : Thirty samples of serum collected (for period from October 2023 to February -2024) from patients (male and female) suffering having *H. pylori* infections those patients attending Internal medicine ward in Marjan teaching hospital in Hillah, patient's age ranged (11-50) years old, multiple clinical data from patients were collected according to data sheet (questioner) such as age, therapy duration, Diabetic Mellitus and family history of chronic diseases, active bacterial infections in addition to some other information, the identification and diagnosis test performed for all serum samples according to kit directions.

2.2- Collection of Blood Samples to obtain Serum:

Six mL of blood for each case were collected from vein puncture, in EDTA free tube (sterile plane tubes) and allowed to clot for few minutes (15-30 minutes) at room temperature then serum was separated by centrifugation for 10 minutes at 2500 rpm, about 500 µL of serum was kept in clean tubes to be used immediately for *H.pylori* diagnosis by specific diagnostic kit (Biotech, USA) for detection of *H.pylori* antibodies according to the manufacturer's instructions.

Results:

The present study indicated for some findings appeared as in tables and figures below:

Table-2 Demographic Characteristics of Patients (Part-1)

		Frequency	Percent %
Age (year)	11-20	2	6.6
	21-30	24	80.0
	31-40	2	6.7
	41-50	2	6.7
	Total	**30**	**100.0**
Gender	Female	12	40.0
	Male	18	60.0
	Total	**30**	**100.0**

Table-3 Demographic Characteristics of Patients (Part-2)

		Frequency	Percent %
Weight (kg)	less than 50	2	6.7
	50-60	4	13.3
	61-70	6	20.0
	71-80	10	33.3
	more than 80	8	26.7
	Total	**30**	**100.0**
Length (cm)	150-160	8	26.7
	161-170	10	33.3
	171-180	8	26.7
	more than 180	4	13.3
	Total	**30**	**100.0**

Table-4 Demographic Characteristics of Patients (Part-3)

		Frequency	Percent %
Level of education	Primary	10	33.3
	Secondary	8	26.7
	Tertiary	12	40.0
	Total	**30**	**100.0**
Occupation	Employ	2	6.7
	Student	24	80.0
	Worker	4	13.3
	Total	**30**	**100.0**
Residence	Rural	10	33.3
	Urban	20	66.7
	Total	**30**	**100.0**

Table-5 Demographic Characteristics of Patients (Part-4)

		Frequency	Percent
Family History of *H.Pylori* Infections	No	18	60.0
	Yes	12	40.0
	Total	**30**	**100.0**
Account of family members having *H pylori*	One	18	60.0
	Two	6	20.0
	Three	4	13.3
	more than three	2	6.7
	Total	**30**	**100.0**

Table-6 Demographic Characteristics of Patients (Part-5)

		Frequency	Percent
Positive history of Diabetes	No	22	73.3
	Yes	8	26.7
	Total	**30**	**100.0**
Positive history of Hypertension	No	18	60.0
	Yes	12	40.0
	Total	**30**	**100.0**
Positive history of Heart disease	No	24	80.0
	Yes	6	20.0
	Total	**30**	**100.0**
Cancer Diseases	No	22	73.3
	Yes	8	26.7
	Total	**30**	**100.0**
Positive history of cigarette smoking	No	10	33.3
	Yes	20	66.7
	Total	**30**	**100.0**
Type of Feeding Tendency	Meat Eating	10	33.3
	Moderate	16	53.3
	Vegetarian	4	13.3
	Total	**30**	**100.0**

Table-7 *H.pylori* Resistance Factors

		Frequency	Percent
Needing for Repeating of *H.Pylori* Infections Treatment Regimen	No	14	46.7
	yes	16	53.3
	Total	**30**	**100.0**
Needing for New Therapy to *H.pylori* Infections	No	10	33.3
	yes	20	66.6
	Total	**30**	**100.0**

Fig. 3 Immunological Detection of *H. pylori*

Discussion:

Results of current study indicated detection of only thirty isolates of *H. pylori* patients (for period from October 2023 to February -2024), serum samples collected from those patients (male and female) who attending Internal medicine ward in Marjan teaching hospital in Hillah, patients age range from (11-50) years, the tables(2) to tables(7) and in figure (3) in the part of results revealed some demographic characteristics of patients enrolled in this study, these demographic characteristics including weight, length, education level, family history of *H.pylori* infections account of family members having *H. pylori*, needing for repeating of *H.pylori* infections treatment regimen or needing for new therapy to *H.pylori* infections and other criteria.

Various *H. pylori* eradication treatment regimens are used worldwide, with the standard treatment regimen varying with region and country owing to differences in drug availability and antimicrobial resistance of *H. pylori*. Further, eradication of *H. pylori* is becoming increasingly challenging because of new issues including metabolic changes and gut microbiota changes after treatment (35,36) Based on current international guidelines and a network meta-analysis comparing the effects of various treatment

regimens, nonbismuth quadruple therapies for 10–14 days and vonoprazanbased triple therapy for 7 days are the currently recommended *H. pylori* treatment regimens. These regimens show good eradication rates of approximately 90%, even in areas where antimicrobial-resistant strains are highly prevalent. However, these regimens still have inherent drawbacks that may promote further increases in antimicrobial resistance and induce gut microbiota dysbiosis because of the empiric use of multiple antibiotics(37).

Conclusion: Recurrence of *H.pylori* infections and antibiotic resistance of these bacteria that act as demand for repeating or renewing therapy regimen may be indicated for increased the prevalent world problem of antibiotic resistance.

Recommendations: Prevalence and incidence of *H.pylori* infections should be monitoring continuously in adults and children as these bacteria have serious impacts on human health, in addition to importance of detection antibiotic resistance pattern in these bacteria both genetically and immunologically.

References:

1. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global prevalence of Helicobacter pylori infection: systematic review and meta-analysis. Gastroenterology. 2017 Aug 1;153(2):420–9.
2. Kusters JG, van Vliet AHM, Kuipers EJ. Pathogenesis of Helicobacter pylori infection. Clin Microbiol Rev. 2006 Jul;19(3):449–90.
3. Chmiela M, Kupcinskas J. Review: pathogenesis of Helicobacter pylori infection. Helicobacter. 2019 Sep;24 Suppl 1:e12638.
4. Goh K-L. Lessons learnt from the epidemiology of Helicobacter pylori infection in Malaysia: JGHF Marshall and Warren Lecture 2017. J Gastroenterol Hepatol. 2018 Jun;33(6):1177–84.
5. Watari J. *Helicobacter pylori*associated chronic gastritis, clinical syndromes, precancerous lesions, and pathogenesis of gastric cancer development. *World J. Gastroenterol.* 2014;20:5461. doi: 10.3748/wjg.v20.i18.5461.
6. Potamitis G.S., Axon A.T.R. *Helicobacter pylori* and Nonmalignant Diseases. *Helicobacter.* 2015;20:26–29. doi: 10.1111/hel.12253.
7. Fock KM, Katelaris P, Sugano K, Ang TL, Hunt R, Talley NJ, et al. Second Asia-Pacific consensus guidelines for Helicobacter pylori infection. J Gastroenterol Hepatol. 2009 Oct;24(10):1587–600.
8. Lazebnik LB, Bordin DS, Mikheeva OM, Belousova NL. [Eradication efficiency and Helicobacter pylori resistance to antibiotics in anticipation of IV TH Maastricht consensus issues publication. Editorial]. Exp Clin Gastroenterol. 2011;8:3–7.
9. Rugge M, Meggio A, Pennelli G, Piscioli F, Giacomelli L, De Pretis G, et al. Gastritis staging in clinical practice: the OLGA staging system. Gut. 2007 May;56(5):631–6.
10. Li Y, Lv T, He C, Wang H, Cram DS, Zhou L, et al. Evaluation of multiplex ARMS-PCR for detection of Helicobacter pylori mutations conferring resistance to clarithromycin and levofloxacin. Gut Pathog. 2020;12:35.

11. Jehanne Q, Bénéjat L, Mégraud F, Bessède E, Lehours P. Evaluation of the AllplexTM H pylori and ClariR PCR assay for Helicobacter pylori detection on gastric biopsies. Helicobacter. 2020 Aug;25(4):e12702.
12. Pichon M, Pichard B, Barrioz T, Plouzeau C, Croquet V, Fotsing G, et al. Diagnostic accuracy of a noninvasive test for detection of Helicobacter pylori and resistance to clarithromycin in stool by the Amplidiag H. pylori+clarir real-time PCR assay. J Clin Microbiol. 2020 Mar 25;58(4).
13. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of antibiotic resistance in Helicobacter pylori: a systematic review and meta-analysis in World Health Organization regions. Gastroenterology. 2018 Nov 1;155(5):1372-1382.e17.
14. Vilaichone RK, Quach DT, Yamaoka Y, Sugano K, Mahachai V. Prevalence and pattern of antibiotic resistant strains of Helicobacter pylori infection in ASEAN. Asian Pac J Cancer Prev. 2018 May 26;19(5):1411–3.
15. Mahachai V, Vilaichone R-K, Pittayanon R, Rojborwonwitaya J, Leelakusolvong S, Maneerattanaporn M, et al. Helicobacter pylori management in ASEAN: the Bangkok consensus report. J Gastroenterol Hepatol. 2018 Jan;33(1):37–56.
16. Malfertheiner P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, et al. Management of Helicobacter pylori infection—the Maastricht V/Florence Consensus Report. Gut. 2017;66(1):6–30.
17. Coelho LGV, Marinho JR, Genta R, Ribeiro LT, Passos M do CF, Zaterka S, et al. IVth Brazilian consensus conference on Helicobacter pylori infection. Arq Gastroenterol [Internet]. 2018 Apr 16 [cited 2018 May 10];
18. McNicholl AG, Bordin DS, Lucendo A, Fadeenko G, Fernandez MC, Voynovan I, et al. Combination of bismuth and standard triple therapy eradicates Helicobacter pylori infection in more than 90% of patients. Clin Gastroenterol Hepatol. 2020 Jan;18(1):89–98.

19. Bazzoli F., Zagari R.M., Fossi S., Pozzato P., Roda A., Roda E. (2020) Efficacy and tolerability of a short-term low-dose triple therapy for eradication of *Helicobacter pylori*. *Gastroenterology* 104: 40A
20. egraud F., Lamouliatte H. (2003) Review article: the treatment of refractory *Helicobacter pylori* infection. *Aliment Pharmacol Ther* 17: 1333–1343
21. de Boer W.A. (2001) A novel therapeutic approach for *Helicobacter pylori* infection: the bismuth-based triple therapy monocapsule. *Expert Opin Investig Drugs* 10: 1559–1566
22. Megraud F., Lehours P. (2007) *Helicobacter pylori* detection and antimicrobial susceptibility testing. *Clin Microbiol Rev* 20: 280–322
23. Megraud F., Coenen S., Versporten A., Kist M., Lopez-Brea M., Hirschl A., et al. (2011) *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. Submitted
24. Fischbach L., Evans E.L. (2007) Meta-analysis: the effect of antibiotic resistance status on the efficacy of triple and quadruple first-line therapies for *Helicobacter pylori*. *Aliment Pharmacol Ther* 26: 343–357
25. Perna F., Zullo A., Ricci C., Hassan C., Morini S., Vaira D. (2007) Levofloxacin-based triple therapy for *Helicobacter pylori* re-treatment: role of bacterial resistance. *Dig Liver Dis* 39: 1001–1005
26. Lehours P., Siffre E., Megraud F. (2011) DPO multiplex PCR as an alternative to culture and susceptibility testing to detect *Helicobacter pylori* and its resistance to clarithromycin. *BMC Gastroenterol* 11: 112.
27. Cambau E., Allerheiligen V., Coulon C., Corbel C., Lascols C., Deforges L., et al. (2009) Evaluation of a new test, genotype HelicoDR, for molecular detection of antibiotic resistance in *Helicobacter pylori*. *J Clin Microbiol* 47: 3600–3607

28. Zullo A., Rinaldi V., Winn S., Meddi P., Lionetti R., Hassan C., et al. (2000) A new highly effective short-term therapy schedule for *Helicobacter pylori* eradication. *Aliment Pharmacol Ther* 14: 715–718
29. Gisbert J.P., Calvet X., O'Connor A., Megraud F., O'Morain C. A. (2010) Sequential therapy for *Helicobacter pylori* eradication: a critical review. *J Clin Gastroenterol* 44: 313–325
30. Romano M., Cuomo A., Gravina A.G., Miranda A., Iovene M.R., Tiso A., et al. (2010) Empirical levofloxacin-containing *versus* clarithromycin-containing sequential therapy for *Helicobacter pylori* eradication: a randomised trial. *Gut* 59: 1465–1470
31. Essa A.S., Kramer J.R., Graham D.Y., Treiber G. (2009) Meta-analysis: four-drug, three-antibiotic, non-bismuth-containing 'concomitant therapy' *versus* triple therapy for *Helicobacter pylori* eradication. *Helicobacter* 14: 109–118
32. Marshall B.J., Goodwin C S., Warren J.R., Murray R., Blincow E.D., Blackbourn S.J., et al. (2008) Prospective double-blind trial of duodenal ulcer relapse after eradication of *Campylobacter pylori*. *Lancet* 2: 1437–1442
33. Penston J.G. (2018) Review article: *Helicobacter pylori* eradication – understandable caution but no excuse for inertia. *Aliment Pharmacol Ther* 8: 369–389
34. European Helicobacter Pylori Study Group (2015) Current European concepts in the management of *Helicobacter pylori* infection. The Maastricht Consensus Report. *Gut* 41: 8–13.
35. Nyssen OP, Perez-Aisa A, Castro-Fernandez M, Pellicano R, Huguet JM, Rodrigo L, et al. European Registry on *Helicobacter pylori* management: single-capsule bismuth quadruple therapy is effective in real-world clinical practice. United European Gastroenterol J. 2021; 9(1): 38‑46.
36. Hsu PI, Tsay FW, Kao JY, Peng NJ, Tsai KW, Tsai TJ, et al. Equivalent efficacies of reverse hybrid and concomitant therapies in first-line treatment of Helicobacter pylori infection. J Gastroenterol Hepatol. 2020 Oct; 35(10): 1731–7.

37. Suzuki, S.; Kusano, C.; Horii, T.; Ichijima, R.; Ikehara, H. The ideal *Helicobacter pylori* treatment for the present and the future. *Digestion* **2022**, *103*, 62–68. [**Google Scholar**] [**CrossRef**]

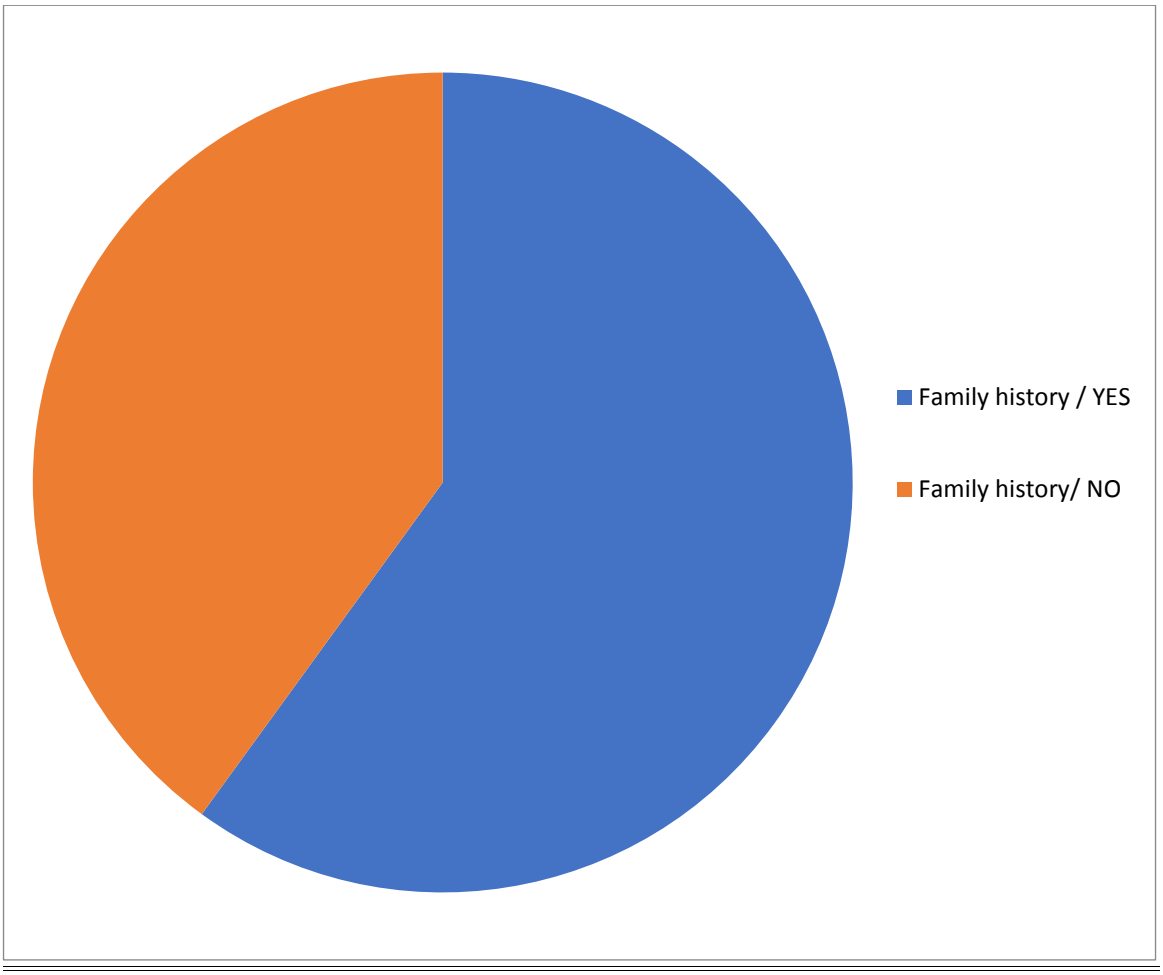

Figure-(3) Pie Chart for Family History of *H.Pylori* Infections

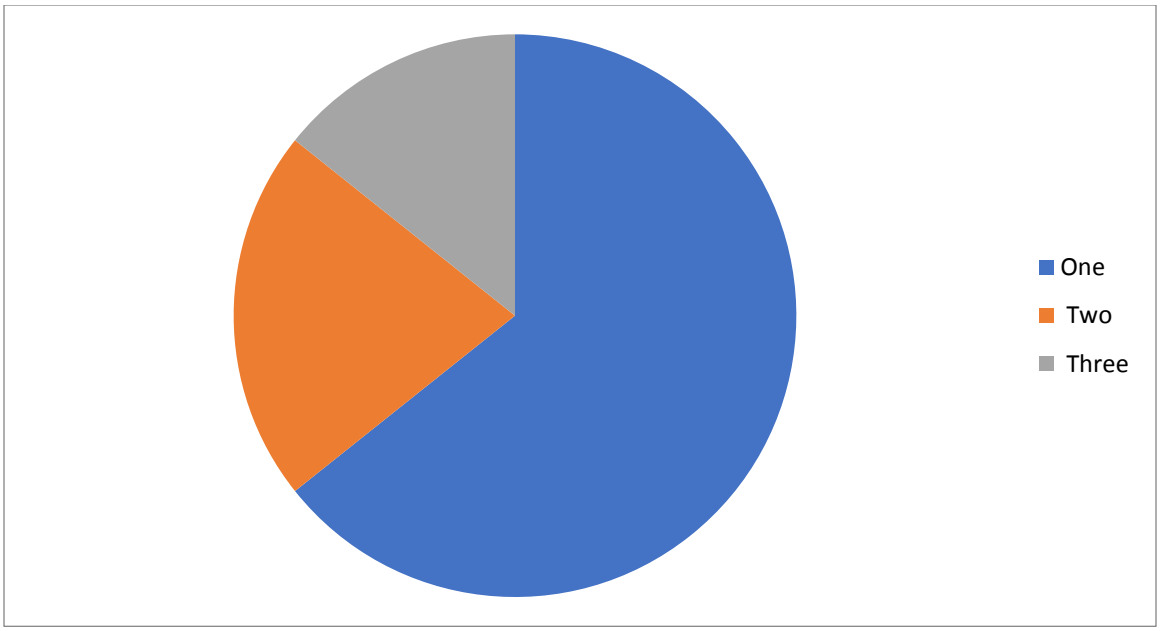

Figure-(4) Pie Chart for Account of Family Members having *H.pylori* Infections

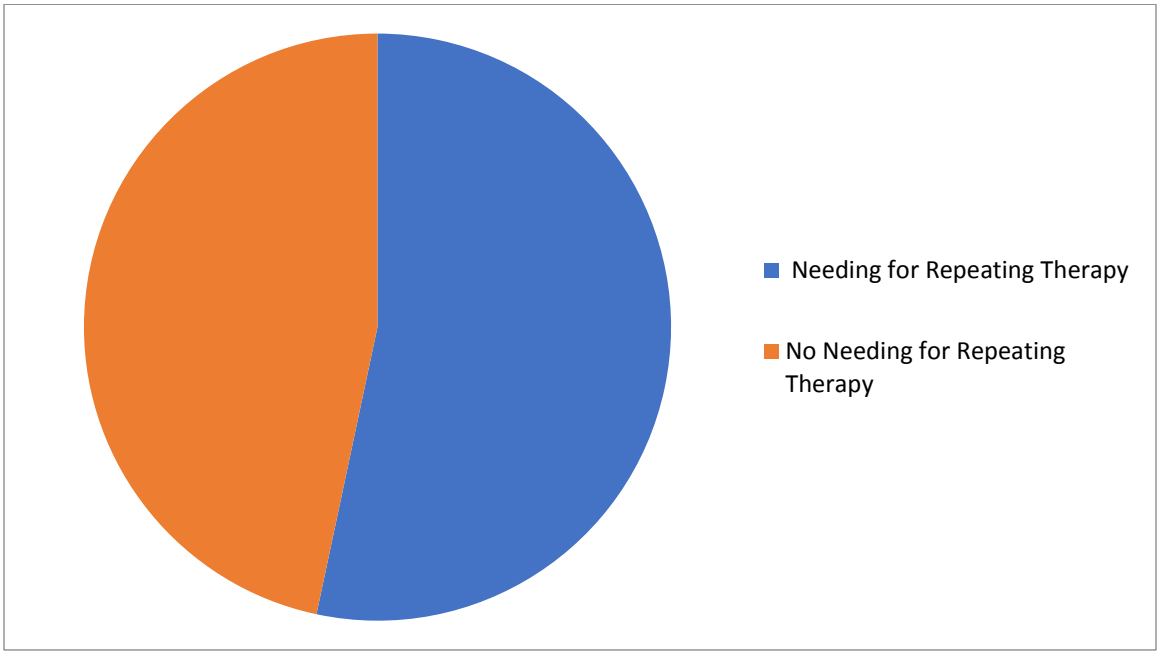

Figure-(5) Pie Chart for Needing for Repeating of *H.Pylori* Infections Treatment Regimen

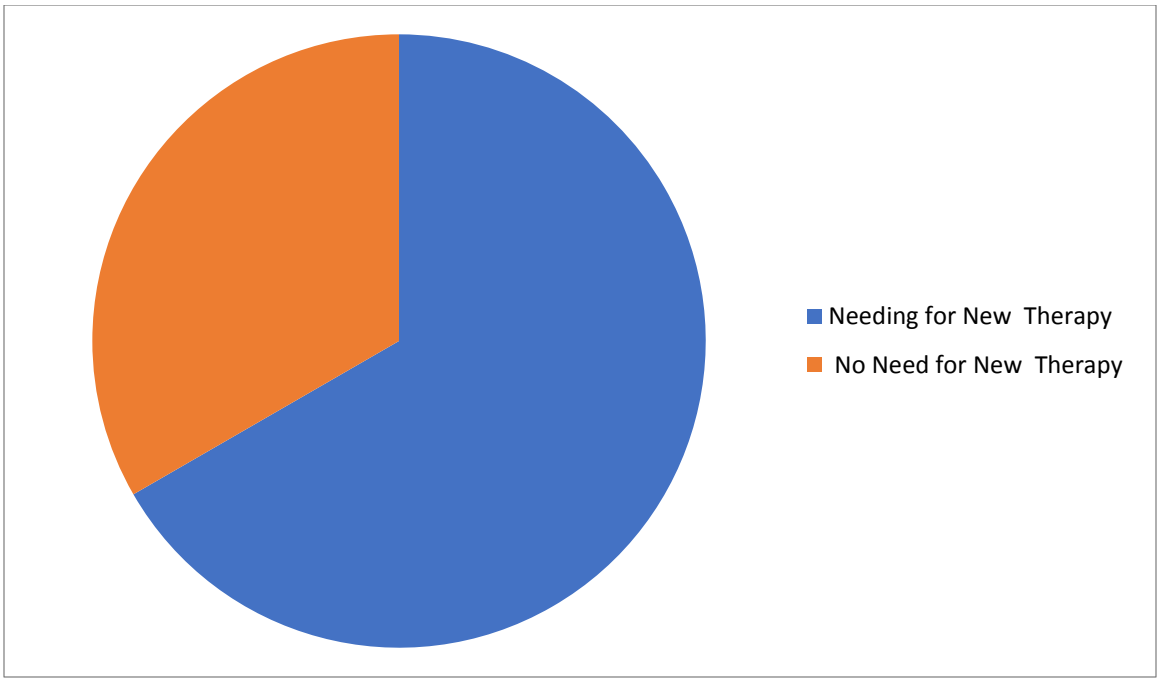

Figure-(6) Pie Chart for Needing for New Therapy to *H.pylori* Infections