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Master's thesis

H. PYLORI INFECTION. CLARITHROMYCIN RESISTANCE IN ERADICATION OF HELICOBACTER PYLORI: PREVALENCE, RISK FACTORS ASSOCIATED WITH RESISTANCE, ADVANCES IN TESTING AND DETERMINING EFFECTIVE TREATMENT REGIMEN

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ABSTRACT

H pylori is associated with various gastrointestinal infections such as chronic gastritis, GERD, functional dyspepsia, peptic ulcer infections, gastric cancer, and MALT lymphomas. H. pylori infection causes duodenal ulcers and gastric ulcers. Providing successful treatment not only successful eradicates the infection but it also improves symptoms, and decreases the rate of recurrences of either duodenal or gastric ulcer [2, 3, 16].

The purpose of the study: to study the factors with leads to development of clarithromycin resistance in eradication of helicobacter pylori: prevalence, risk factors associated with resistance, advances in testing and determining effective treatment regimen

Research Assignments.

1. To study the Reason for resistance
2. To Analyze the Prevalence of Resistance to Clarithromycin
3. To identify the Factors leading to treatment failure
4. To determine the Bacterial Resistance rising prevalence of antibiotic resistance in eradicating the infection

Object of research. The patients with gastrointestinal disease caused by H.Pilory infection.

Subject of research. Prevalence of Resistance to Clarithromycin, Factors leading to treatment failure, Factors in clarithromycin resistance, as well as the organization of the nurse's work on the prevention and care of patients with gastrointestinal disease caused by H.Pilory infection.

Research methods: general clinical methods of studying patients used in gastroenterology for various age categories of patients (laboratory and instrumental research methods), as well as analytical methods and statistical methods.

Scientific and practical significance of the study. With the help of this scientific study the main factors that lead to development of clarytromicine resistance were identify. Factors leading to treatment failure was studied. What happens if H. Pylori is resistant to antibiotics. Treatment-related factors was analyze .

Conclusions:

1. H pylori is associated with various gastrointestinal infections such as chronic gastritis, GERD, functional dyspepsia, peptic ulcer infections, gastric cancer, and MALT lymphomas. H. pylori infection causes duodenal ulcers and gastric ulcers. Providing successful treatment not only successful eradicates the infection but it also improves symptoms, and decreases the rate of recurrences of either duodenal or gastric ulcer.
2. In the United States, the current first line treatment for eradicating H. pylori comprise of various antibiotic procedures such as PPI, amoxicillin and clarithromycin for duration of 10-14 days, inpatient without PCN allergy and inpatient with PCN allergy it has been recommended that amoxicillin should be substituted with metronidazole. Perhaps, bismuth-based quadruple therapy is used as an alternative in case of failure of clarithromycin therapy. In addition, bismuth-based quadruple therapy can also be used as a primary treatment regimen in patients with PCN allergy or if the patient is known to have clarithromycin resistance. Different researches has indicated that H. pylori antibiotic resistance has gradually increased around the world.

However, the rate of antibiotic resistance and the type of antibiotic resistances vary from country to country. In addition, American has the highest clarithromycin resistance, followed by Asia and then Europe. The study also illustrated that Africa has among the highest Metronidazole resistance, followed by America, then Asia and finally Europe. For the European nations, Spain reported the highest rate of clarithromycin resistance and the lowest clarithromycin resistance among the European countries were Sweden and the Netherlands respectively.

3. 3. The two most important factors in predicting treatment failure were antibiotic resistance and poor complaints. It has been implicated that clarithromycin resistance was due to point mutation in the peptidyltransferase domain V of the 23S rRNA. From all the subsets of point mutation, three most common point mutations were A2143G, A2142G and A2142C, and A2143G being the most common point mutation that is primarily responsible for clarithromycin resistance in H. pylori Strain. The rising rate of clarithromycin resistance in the H. pylori strain is largely due to widespread use of clarithromycin for treatment other than H. pylori infection (i.e clarithromycin for respiratory infections) in the general population. In addition, H. pylori eradication rate has also decreased due to Metronidazole resistance as well. It has been determined that metronidazole resistance in women is greater than men. Like clarithromycin resistance,

metronidazole resistance has also occurred largely due it widespread use other than H. pylori infection treatment (i.e metronidazole for gynecological infections, parasitic infection, and sexually transmitted disease). Besides the antibiotic resistance, poor compliance was also an issue in regard to treatment failure. A Study on Failure of Helicobacter pylori eradication in an ambulatory population conducted by Wermeille J, Deterding JP and Cunningham M, illustrated that patient compliance played an important role in successful eradication. However, a significant proportion of the treatment failure could not be explained by this study and thus alternative factors such as antibiotic resistance needed to be taken into consideration.

4. 4. In the United States, the current recommended guidelines for initial H. pylori treatment regimen is clarithromycin-based triple therapy. If clarithromycin-based triple therapy fails or a patient has PCN allergy, then bismuth-based quadruple therapy has been recommended. However, due to clarithromycin resistance and decreased eradication yield with clarithromycin-based triple therapy, several studies have looked at different antibiotics in order to find effective treatment regimens, with good patient compliance with minimal antibiotic side effects. Two possible treatment regimens shows to be promising, but need further studies in the United States prior to getting validated in North America. The first treatment regimen was composed of PPI and amoxicillin for 5 days followed by PPI,

clarithromycin and tinidazole for an additional 5 days. This regimen achieved eradication rates superior to clarithromycin-based triple therapy and had a better patient compliance rate. The second regimen was levofloxacin-based triple therapy (PPI, levofloxacin and amoxicillin for 10 days) showed to be more effective and better tolerated than bismuth quadruple therapy in patients with persistent H. pylori infection. But until the two regimens get validated, clarithromycin-based triple therapy remains the first-line regimen for H. pylori eradication.

5. 5. Also, individual with persistent H. pylori with treatment failure from first-line treatment regimen or second-line treatment regimen, the clinician should prompt the patient to have repeat endoscopy with culture and susceptibility testing done to determine which antibiotic the patient is resistant to and to which antibiotic the patient is susceptible too. The H. pylori eradication rate had significantly improved when antibiotic therapy was given on the basis of the results of an anti-microbial susceptibility test. However, currently no gold standard method has been proposed for testing H. pylori susceptibility to antibiotics. Though culture or PCR are the primary means by which sensitivity can be determined, neither of the tests are widely available for clinical use in the United States. Thus currently, no guidelines have been placed, in which it would implement clinicians to use PCR based

techniques to determine clarithromycin resistance H. pylori strain individuals with treatment failure.

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INTRODUCTION

Helicobacter pylori (H. pylori) is a small curved, micro-aerobic, gram-negative, rod-shaped bacterium that produces urease, which hydrolyzes the gastric luminal urea to form ammonia. Ammonia helps neutralize gastric acid and create a protective cloud around the organism, enabling it to penetrate the gastric mucus layer, colonize the gastric mucous layer, or adhere to the stomach's epithelial lining. H. Pylori infection is recognized as one of the causal factors in the development of chronic gastritis, gastroesophageal reflux disease (GERD), functional dyspepsia (F.D.), and peptic ulcer disease (PUD)- which is having either gastric or duodenal ulcers in the stomach or the duodenum respectively. In addition, H. pylori infection is associated with the development of gastric cancer and mucosa-associated lymphatic tissue (MALT) lymphomas. The International Agency for Research on Cancer has categorized H. pylori as a group 1 carcinogen. Stomach cancer is the fifth most common cancer and the third-highest mortality ratio. Therefore it is very crucial to eradicate this pathogen for the prevention of gastric cancer.

H. pylori cause more than 95 percent of duodenal ulcers and up to 80 percent of gastric ulcers (4, 5, 12, 16). Individuals who are infected with H. pylori are most commonly asymptomatic. The infection is likely to occur in childhood and may remain in the patient system for many years without showing any symptoms. Common symptoms are epigastric burning, abdominal distention or bloating,

belching, nausea, flatulence, and halitosis (12). In addition, some of the presenting symptoms of either PUD or GERD include epigastric pain or discomfort that is burning in nature, which may be localized or may be radiated to the sub-sternal region and can be associated with nausea, vomiting, loss of appetite, vomiting blood (hematemesis) and/or black tar stool (melena). Such symptoms can occur due to H. pylori being the underlying cause of developing a duodenal or gastric ulcer or the development of gastritis. (4, 12).

According to the Center for Disease Control and Prevention (CDC), it is estimated that the prevalence of H. pylori is 70% in developing countries and 30-40% in the United States and other industrialized countries (16). The annual incidence of new H. pylori infection in industrial countries is approximately 0.5 per 100 persons of the susceptible population compared with three or more per 100 persons in developing countries (2). In the United States, H. pylori are more prevalent among older adults, African Americans, Hispanics, and lower socioeconomic groups (12,16). Diagnosis of H. pylori infection can be determined by either a noninvasive or invasive approach. Noninvasive approaches include fecal antigen detection, serologic testing, and urea breath testing. The invasive procedure involves endoscopic biopsy evaluation using rapid urease testing or histological examination (12,14). However, it is unclear whether eradication of this bacterium improves symptoms in patients with non-ulcer dyspepsia. Still, there is strong evidence that eradicating this bacterium enhances healing and reduces the

risk of recurrence or rebreeding in patients with duodenal or gastric ulcers. For patients who have dyspepsia-like symptoms, it had been recommended that individuals should be tested for H. pylori using a noninvasive method if there is no clinical evidence suggesting the need for endoscopic evaluation. Such individuals who tested positive for H. pylori should be treated. This "test and treat" approach not only improves symptoms but has shown to be cost-effective compared with other strategies (2, 12).

CHAPTER 1

H PYLORI INFECTIONS – SOURSE OF PROBLEM, TREATMENT AND PROFILAXY (LITERATURE REVIEW)

1.1. Who gets H Pylori infections?

H. pylori infection mainly occurs in children. In the United States, 5% of the total number of children under ten years are infected with H. pylori infection. However, it is not yet known the exact way in which people get infected by H. pylori. However, clinical trials have shown that it is transmitted from one person to another through direct psychical contact such as saliva exchange through kissing, vomiting, and fecal matter, among others. Some scholars have shown that H. pylori are spread through water and food contaminated with H. pylori. However, since it exhibits itself as a dormant infection, many people don't realize that they have it until it gets worse in their body unless it is diagnosed early. In many cases, the bacteria that causes H. pylori is perceived to be present at around 50-75% of the total world population. However, this bacteria does not lead to illness n many people as it is always dormant, and it's common in developing countries due to poor sanitation and crowding in urban centers. Due to the low immune system among the children, overcrowding and poor sanitation can lead to increased prevalence and infections of H. pylori across the world.

1.2. Who is at risk for H. pylori infection?

In many cases, H. pylori are spread from one person to another as it's a contagious infection. It is found in saliva, teeth, and poops. Kissing is another approach in which H. pylori can transfer. It can also be transferred from the unwashed hands to another person or either contaminated water or food. This implies that physical contact between infected people can lead to the spread of H. pylori. With this understanding, people who live in crowded places are at high risk of getting H. pylori as the chances of physical contact are high. People living without a reliable, clean water supply are also at risk of contracting H. pylori. Having a reliable supply of clean water helps reduce the risk of H. pylori. Additionally, living in developing countries increases the chances of contracting H. pylori due to overcrowding and unsanitary living conditions and living with someone who has an H. pylori infection. If someone you live with has H. pylori infection, you are more likely to get it as well. Age is also another factor that increases the risk of contracting H. pylori. More than 50 % 0f people in the U.S. are over 50 years old. Lastly, race and ethnicity also influence the rate of H. pylori contracting. Almost half of African Americans have H. pylori.

1.3. H. pylori testing indication:

In general, H. pylori testing is indicated for patients with clinical manifestations of H. pylori infection, including a history of or active PUD or

MALT Lymphoma. In addition to the gastrointestinal manifestation of H. pylori testing in nondiagnostic diseases such as unexplained Iron Deficiency Anemia (IDA) and Idiopathic thrombocytopenia purpura (ITP). Generally, H. pylori testing is performed if the clinician plans to provide treatment for positive medical results. There are two significant approaches used to test and indicate H. pylori. These include using MALT lymphoma and using active peptic ulcer infections or assessing the patient's past history to ascertain the prevalence of H. pylori-related infections.

Nonendoscopic
Stool (fecal) antigen test Urea breath test Serology/antibody testing (quantitative and qualitative)
Endoscopic
Culture Histology Rapid urease testing Polymerase chain reaction
H pylori: Helicobacter pylori. *Source: References 2, 5.*

Table 1: Tests for H. pylori are categorized as endoscopic or non-endoscopic.

1.4. How are H. pylori diagnosed?

There are many different ways to diagnose H. pylori.

- Blood test.

Blood test checks for H. pylori antibodies. This means that the patient is currently infected or has been infected in the past.

- Stool culture.

The stool antigen test is the most common stool test to detect H. pylori. Proton Pump inhibitors (PPI), antibiotics, and Pepto-Bismol can interfere with the accuracy of results. Thus doctors recommend stopping those meds for 1-2 weeks. Also, a stool polymerase chain reaction (PCR) test can detect H. pylori infection in stool and show resistance to antibiotics. This test is very expensive and might not be available in every clinic.

- Breathe Tests.

During a breath test, you swallow a pill or liquid that has a tagged carbon molecule. In a patient who has H. pylori, carbon is released when the solution is broken down in the stomach. Then the patient exhales into the bag, where they use a special device to detect the carbon molecule. Similar to stool tests, PPI, antibiotics, and Pepto-Bismol can impact the accuracy of this test. If the patient was diagnosed with H. pylori and received full treatment, he or she will need to wait 4 weeks for a breath test to confirm eradication of infection.

- Upper Endoscopy -EGD (esophagogastroduodenoscopy).

Upper endoscopy is an invasive procedure that checks the esophagus, stomach, and duodenum lining. This test helps us to investigate gastric ulcers, gastritis, or cancer. Biopsy samples are analyzed. H. pylori.

It is prudent to note that conducting a test, clinical situation, and cist are three significant considerations used to select the diagnostic tests among the patients suspected to have H. Pylori. Even though there is a standard measure used to identify H. pylori, the urea breath test is considered the standard and appropriate method preferred among the patients without underlying symptoms since it's not invasive and specific. Additionally, culture is considered as another approach used to diagnose H. pylori. Although it's not widely used, culture is used to enhance susceptibility testing, and it's used in areas which has high clarithromycin resistance or in case of failure of therapy. This is because the first-line regimen failure implies that there is a 60-70% chance of clarithromycin resistance (5).

1.5. Treatment:

For the patients who test active for H. pylori infection, treatment should be described with immediate effect to prevent more spread of infection within the body. The use of antibiotics combined with acid reduction proton pump inhibitors are the two common approaches that can be used to treat H. Pylori. Some of the common antibiotics that can be administered to patients with H. pylori infections include amoxicillin, clarithromycin, and metronidazole, among others. However, allergies and adherence to the prescriptions determine the success of the treatment process, and hence patients should ensure they are in close contact with healthcare providers to ensure effective medical treatment for this infection.

H pylori Treatment Regimens

Therapy	Dosing	Duration (days)[a]	Eradication (%)
First-Line Options			
Clarithromycin triple	PPI[b] bid Clarithromycin 500 mg bid Amoxicillin 1 g bid *or* Metronidazole 500 mg tid	14	70-85
Bismuth quadruple[c]	PPI bid Bismuth subcitrate 120-300 mg/ bismuth subsalicylate 300 mg qid Metronidazole 250 or 500 mg qid Tetracycline 500 mg qid	10-14	75-90
Nonbismuth-based quadruple	PPI bid Amoxicillin 1 g bid Clarithromycin 500 mg bid Metronidazole 500 mg bid	10-14	90
Alternative or Salvage			
Sequential	PPI bid and amoxicillin 1 g bid, then: PPI bid Clarithromycin 500 mg bid Metronidazole 500 mg bid	5-7, then 5-7 (total 14)	>84[d]
Hybrid	PPI bid + amoxicillin 1 g bid, then: PPI bid + clarithromycin 500 mg bid + amoxicillin 1 g bid + metronidazole 500 mg bid	7, then 7 (total 14)	88.6[d]
Levofloxacin-Containing (Alternative or Salvage)			
Levofloxacin-based	PPI bid Levofloxacin 500 mg qd Amoxicillin 1 g bid	10-14	79-81[d]
Levofloxacin sequential	PPI[b] bid + amoxicillin 1 g bid, then: PPI bid + levofloxacin 500 mg qd + metronidazole 500 mg bid	5-7 then 5-7 (total 14)	83.6-87.4[d]
LOAD (levofloxacin, omeprazole, Alinia, doxycycline)[e]	PPI (double dose qd) Levofloxacin 250 mg qd Nitazoxanide (Alinia) 500 mg bid (or metronidazole 500 mg bid) Doxycycline 100 mg qd	7-10	89[d]

Table 2: H. pylori treatment regimen

Based on the above table, CGG on the management of H. pylori infection, the U.S. recommends the primary treatment regimen for the H. pylori, which includes PPI, amoxicillin, clarithromycin as well as metronidazole, among others. The effectiveness of these treatment approaches is considered to be between 10-14 days after administration. It is also believed that the increase of first-line antibiotics is an important aspect that can help to eradicate H. pylori infections.

A research study by (4) involved a meta-analysis of 25 randomized control trials aimed to evaluate the eradication process of H. pylori with clarithromycin resistance. The study investigated 2100 patients who had experienced a 12 month ulcer remission rate, which comprised 95% of the gastric ulcers and 97% of duodenal ulcers among the patients who had successfully eradicated H. pylori. The study showed that the patients with gastric ulcers and who had experienced duodenal problems are 655 likely to experience a great challenge of H. pylori eradication (4). However, various researches have shown that because of the continual increase of H. pylori resistance to clarithromycin, the use of PPI has become less effective, which in turn results in treatment failure for the eradication process of H pylori and also subsequent treatment regimens (11, 14).

1.6. Current Guidelines in Helicobacter pylori Eradication

In the United States, H. pylori infections and their prevalence differ from one geographical location to another as it affected by demographic issues. In the U.S., the recommendable and standard therapies that can be used for H. pylori infections includes PPI, clarithromycin, amoxicillin, and metronidazole, and its effective for 14 days after administration (4, 12, 14, 16). For the patients with underlying comorbidities related to penicillin allergies, metronidazole is in many cases substituted for amoxicillin. According to the international guidelines, the recommended treatment duration for the H. pylori treatment regimen has been

implemented to at least 7 days. However, in the United States, a treatment duration of 10-14 days has typically been employed. This implies that any details concerning H. pylori are found in table 3 below (4).

According to the ACG about H. pylori management, various studies have investigated the effectiveness of combining PPI, clarithromycin, and amoxicillin in the eradication of H. pylori, and the results show that 8 out 10 days of therapy resulted in similar eradication rates. The intention to treat eradication rate for the first one week was 78% as compared to 80% for the 10 days of regimens administration. Additionally, a meta-analysis study that comprised of 7 studies involving over 900 patients determined that 14 day treatment duration provided superiority over 7 day treatment duration in H. pylori eradication rates. Regarding bismuth quadruple therapy, the rate of eradication is same to the clarithromycin therapy (4).

Regimen	Duration	Eradication Rates	Comments
Standard dose PPI b.i.d. (esomeprazole is q.d.), clarithromycin 500 mg b.i.d., amoxicillin 1,000 mg b.i.d.	10–14	70–85%	Consider in nonpenicillin allergic patients who have not previously received a macrolide
Standard dose PPI b.i.d., clarithromycin 500 mg b.i.d. metronidazole 500 mg b.i.d.	10–14	70–85%	Consider in penicillin allergic patients who have not previously received a macrolide or are unable to tolerate bismuth quadruple therapy
Bismuth subsalicylate 525 mg p.o. q.i.d. metronidazole 250 mg p.o. q.i.d., tetracycline 500 mg p.o. q.i.d., ranitidine 150 mg p.o. b.i.d. or standard dose PPI q.d. to b.i.d.	10–14	75–90%	Consider in penicillin allergic patients
PPI + amoxicillin 1 g b.i.d. followed by: PPI, clarithromycin 500 mg, tinidazole 500 mg b.i.d.	5 5	>90%	Requires validation in North America

PPI = proton pump inhibitor; pcn = penicillin; p.o. = orally; q.d. = daily; b.i.d. = twice daily; t.i.d. = three times daily; q.i.d. = four times daily.
*Standard dosages for PPIs are as follows:
lansoprazole 30 mg p.o., omeprazole 20 mg p.o., pantoprazole 40 mg p.o., rabeprazole 20 mg p.o., esomeprazole 40 mg p.o.
Note: the above recommended treatments are not all FDA approved. The FDA approved regimens are as follows:
1. Bismuth 525 mg q.i.d. + metronidazole 250 mg q.i.d. + tetracycline 500 mg q.i.d. × 2 wk + H_2RA as directed × 4 wk.
2. Lansoprazole 30 mg b.i.d. + clarithromycin 500 mg b.i.d. + amoxicillin 1 g b.i.d. × 10 days.
3. Omeprazole 20 mg b.i.d. + clarithromycin 500 mg b.i.d. + amoxicillin 1 g b.i.d. × 10 days.
4. esomeprazole 40 mg q.d. + clarithromycin 500 mg b.i.d. + amoxicillin 1 g b.i.d. × 10 days.
5. Rabeprazole 20 mg b.i.d. + clarithromycin 500 mg b.i.d. + amoxicillin 1 g b.i.d. × 7 days.

Table 3: First-Line Regimens for Helicobacter pylori Eradication

CHAPTER 2

2.1. Prevalence of Resistance to Clarithromycin

Various researches have shown that the eradication rate for H. pylori using the first-line treatment using PPI, clarithromycin, and amoxicillin has reduced to 75% due to the increase of clarithromycin resistance (4). Additionally, clarithromycin-based triple therapy is faced with a great problem of high resistance rate to metronidazole. For instance, a multi-center study based on the U.S. from 1994-1999 showed that that the antibiotic resistance rate among the patients with H. pylori are likely to experience strain by 38% for the metronidazole, 15% for the clarithromycin, 4% for antibiotics and 1.5% for amoxicillin. The same author collected another additional data e from 1999 – 2002 and showed that the resistance rate was 28% for metronidazole, clarithromycin (15%), antibiotics (5%) and amoxicillin (1%) (4). Based on such data, it appears the clarithromycin resistance rate has increased over the years whereas the amoxicillin and metronidazole resistance rate has slightly increased but relatively remain stable. Moreover, a different study illustrates that high clarithromycin and metronidazole resistance rates were found in Europe, America, and Asia (7).

According to World H. pylori Antibiotic Resistance: a study by Vincenzo De Francesco and colleagues, illustrated the resistance rate of the overall H. pylori antibiotic were 26.7% for metronidazole, 17.2% for clarithromycin, 16.2% for levofloxacin, 11.2% (95% CI: 9.6-12.7%) for amoxicillin, 9.6% for multiple

antibiotic, 5.9% for tetracycline, and 1.4% for rifabutin. (6). Details regarding resistance rates on antibiotics in various geographical areas is indicated in table 4 and 5 below

Area	Amoxycillin	Clarithromycin	Metronidazole	Tetracycline	Levofloxacin	Multidrugs
America	8/352	118/402	177/401	11/393	NA	53/352
	(2.2%)	(29.3%)	(44.1%)	(2.7%)		(15.0%)
Africa	113/172	NA	159/172	58/132	0/40	NA
	(65.6%)		(92.4%)	(43.9%)	(0.0%)	
Asia	60/517	1,544/8,139	192/517	11/456	106/908	21/252
	(11.6%)	(18.9%)	(37.1%)	(2.4%)	(11.6%)	(8.3%)
Europe	3/599	352/3156	420/2,459	14/599	148/614	204/2,272
	(0.5%)	(11.1%)	(17.0%)	(2.1%)	(24.1%)	(8.9%)
Overall	184/1,640	2,014/11,697	948/3,549	94/1,580	254/1,562	278/2,876
	(11.2%)	(17.2%)	(26.7%)	(5.9%)	(16.2%)	(9.6%)

Table 4: Antibiotic resistance rates in different continental areas.

	Year	Patients	AMO (%)	CLA (%)	MTZ (%)	TET (%)	LEV (%)	RIF (%)	Multidrug (%)	Ref
Europe										
Bulgaria	2004-08	266	1.1	15.4	24	4.9			4.9	9
Denmark	2001-03	81		11	28					10
Ireland	2005-06	45		8.8	20				2.2	12
Italy	2003-06	146		37.6						13
Italy	2001	156		24.3						14
Italy	2004-06	255		11.0	20		10.6		10.5	15
Italy	2003	300		12.5	23.9				4.3	16
Germany	2003-06	1585						1.4		17
Germany	2006-07	1118							15.1	18
Spain	2002-06	101		49.2	32.8					19
Sweden	2002	333	0	1.5	14.4	0.3			0.6	20
The Netherlands	1997-02	1123		1	14.4					21
Turkey	2002-03	87		27.5						22
Turkey	2003-04	110		48.2						23
Turkey	2005-06	92		40.5						24
Turkey	2006-07	61		16.4						25
United Kingdom	2008	255					7.5	6.6		11
Asia										
China	2000	41		32						30
Japan	1996-08	3521		20.6						26
Japan	2001-04	507					14.9			27
Japan	2002-05	3707		22.7						28
Japan	2002-07	61 (children)	0	40.7	14.8					29
Hong Kong	2004-05	191					2.6		8.9	32
Korea	2003-05	113	8.8	12.4	49.6	8.8	12.3			31
Malaysia	2005-07	120		2.1						35
Taiwan	1998-07	210	1	9.5	27.6	0.5	11.9			33
Taiwan	2004-05	133	36.1	13.5	51.9	0				34
North America										
Alaska	1999-03	352	2.2	31	44	0			15	36
South America										
Chile	2005-06	50		20	44.9	26.8				37
Africa										
Senegal	1999-00	40	0		90		0			38
Cameroon	2006	132	85.6	44.7	93.2	43.9				39

Table 5: Antibiotic resistance rates in different countries

Both Table 4 and Table 5 have been extracted from Worldwide H. pylori Antibiotics Resistance: (6). As shown in Table 4, the overall clarithromycin resistance was detected in 2,014 out of the 11,697 cases. Furthermore, out of the four continental areas (America, Africa, Asia, and Europe), America had the highest clarithromycin resistance (118/402, 29.3% 95% CI: 24.9-33.8), followed

by Asia (1,544/8,139, 18.9%; 95% CI: 18.1-19.8) and then Europe 352/3156, 11.1%; 95% CI: 10.0-12.2). Table 2A also illustrated that Africa is among the highest Metronidazole resistance, followed by America, then Asia, and finally Europe. According to Table 5, Spain recorded the highest rate among the European countries with a clarithromycin resistance rate of 50% (49/101, 49.2% 95% CI: 38.7-58.2). Also, based on Table 5, Japan recorded the highest clarithromycin resistance among the Asian countries (25/61, 40.7% 95% CI: 28.5-53.3). Among the European countries, Sweden and Netherland had among the lowest clarithromycin resistance (5/333, 1.5% 95% CI: 38.7-58.2) and 11/1223, 0.8% 95% CI: 0.3-1.4) respectively (6).

2.2. What happens if H. Pylori is resistant to antibiotics?

The persistence and prevalence of H. pylori infection is considered to cause a huge damage to the human body and especially gastric mucosa, which in turn results in serious infections such as peptic ulcers, MALT lymphoma, anemia as well as gastric adenocarcinoma in around 30%. H. pylori is perceived as the most common cause of peptic ulcers which comprise of a secrete enzyme known as urease which is responsible for converting urea to ammonia. The role of this ammonia is to protect the stomach from releasing the stomach acid that causes peptic ulcers. When H. pylori replicates, it consumes the stomach tissues and hence leading to the growth of gastric and peptic ulcers. In 2017, Due to the significant

global prevalence of H. pylori and increasing antibiotic resistance and complications, the WHO recognized H. pylori as a high-priority pathogen.

2.3. Factors leading to treatment failure

2.3.1. Lack of patient compliance.

One of the significant factors that leads to treatment failure among the patients with H. pylori infections is poor compliance to the treatment procedure. Various studies have shown that lack of close coordination and engagement between clinicians and patients can result in poor medical intervention. Perhaps, it has been noted that in many cases, H. pylori is experienced among the patients with inactive peptic ulcers, which shows that it's prevalent in the dormant cells/enzymes. This implies that of its not diagnosed early enough it can be challenging it treat it at the advanced stages and hence leading to treatment failure. Additionally, if patients are not in compliance with the medical guidelines and medical prescriptions, the chances of H. pylori multiplication is high. For instance, for patients who take over 60% of the stipulated medications are likely to experience less prevalence of H. pylori. This in turn, shows that compliance to the medical treatment and prescriptions plays an essential role in eradicating H. pylori.

Ineffective penetration of antibiotics into the gastric mucosa, antibiotics inactivation by the low stomach ph.

Another significant factor that leads to treatment failure is ineffective penetration of antibiotics into the gastric mucosa, which is an important aspect of ant-helicobacter pylori treatment. Omeprazole decreases gastric juice and hence increases intragastric pH. For instance, a study done by (7) aimed to investigate the chemical stability of the clarithromycin in human gastric juice before and after the first week of omeprazole administration. The lab experiment for the study showed that the gastric juice sample was obtained from the volunteers who had tested negative for H. pylori. The results showed that all the three antibiotics examined were stable in an aqueous solution of pH 4.0-7.0, pH 5.0-8.0, and pH 2.0-7.0, respectively. However, at pH 2.0 the antibiotics degraded rapidly. Thus this shows that the co-administration of omeprazole with antibiotics is likely to increase its stability.

2.3.2. Bacterial Resistance rising prevalence of antibiotic resistance in eradicating the infection (3, 13).

Various clinical experiments have shown a positive correlation between the bacteria resistance to antibiotics and the failure of H. pylori eradication. For instance, for the patients experiencing metronidazole, the eradication rate is around 30% lower than those infected with metronidazole when treated with triple therapy. Additionally, the same results are recorded when patients are using PPI and clarithromycin. This implies that H. pylori resistance to the clarithromycin is low as compared to metronidazole globally. It is also prudent to note that the presence

of bacterial resistance to clarithromycin is 100% likely to result in treatment failure when done using dual therapy. This implies dual treatment is not an effective approach to eradicate H. pylori and hence it should not be used.

There is also secondary bacterial resistance to H. pylori in patients who failed previous eradication treatment. In one of the studies, metronidazole resistance was experienced in 30% of the patients investigated before the eradication treatment procedure was done. The study also showed that Clarithromycin resistance increased from 33% to 70% before treatment and after failed treatment. This implies that a previous treatment failure can have a great influence on the increase of the antibiotic resistance among patients suffering from H. pylori.

2.3.3. Treatment-related factors.

The components of the treatment regimen plays an essential role in enhancing the eradication of H. pylori among the patients. These includes the selected drugs, medication doses prescribed to the patient, duration of treatment and therapy as well as dosing frequency, among others. For example, triple therapy using Proton pump inhibitors (PPI) effective is considered to be more effective as compared to the use of dual therapy. Additionally, the PPI triple approach helps eradicate more H pylori infections when administered within the first two weeks of infection. Lastly, administering PPI twice a day can be more effective than once in

a day when combined with clarithromycin and amoxicillin. In conclusion, to get better results to eradicate H. Pylori, it's important to include full doses of medications, adequate treatment duration, and optimal dosing frequency.

From all of the factors stated above, the most important predictor of treatment failure includes antibiotic resistance and poor compliance (3, 4). Various studies have shown that eradication rates obtained during the first line using PPI, clarithromycin, and amoxicillin improve the eradication rate by 70% and reduce the clarithromycin resistance by 30% (4). Clarithromycin resistance is not the only anti-microbial resistance that is rising in prevalence. Resistances of anti-microbials such as metronidazole, tetracycline levofloxacin, and amoxicillin have also been noted to be increasing as well. However, the major factor in standard triple therapy (clarithromycin-based triple therapy) is the increased number of both clarithromycin and metronidazole resistance rates in H. pylori eradication (4, 10,11).

2.3.4. Factors in clarithromycin resistance

Anti-microbial resistance, particularly clarithromycin resistance in H. pylori infection eradication, can result from various H. pylori strains and gene mutation resulting in treatment failure. One of the significant role of clarithromycin is to bind together the peptidyltransferase region that surrounds the 23S rRNA and prevent replication and synthesis of the protein enzymes. Clarithromycin resistance

within H. pylori is seen because of the point mutation that is embedded within the peptidyltransferase domain V of the 23S rRNA (3, 7, 9, 10). The H. pylori comprise of two genes of 23S rRNA where the most common mutation is A to G transition at positions 2143 (A2143G), while various point mutations are positioned A2142G, A2144G, and T2182C. In addition, several other reports have also been identified. They have indicated that different mutations such A2115G, A2144G, A2142C, A2142G, A2144T, A2223G, C2147G, C2195T, C2694A, G2115A, G2141A, T2182C, T2190C, T2717C have also been implicated in association with clarithromycin resistance (3, 9,11). All the subsets of point mutation that were named above, resulted to 90% of all clarithromycin resistance cases in H. pylori strains isolated in Western countries (7,10). It was also determined that the A2143G strain also has predominantly been noted to be seen in Europe and Japan as well (3). The Saudi Journal of Gastroenterology also confirmed that joint mutation is A2143G which accounts for (30/32: 93.7%) to the occurrence of clarithromycin resistance among the H. pylori patients (1).

Clarithromycin resistance is also due to H. pylori genotype variations. (cytotoxin-associated gene A [cagA]) and vacuolation-associated gene a [vacA]) are the two genes that are considered to be a major factor related to H. pylori severity (3,5,8). While assessing the vacA gene, it was found that the combination of s2m1 mosaics is equivalent to s1/m1 and s2m2 and their severity and prevalent was equal (10). The same study also showed that the s2m2 strains are resistant to

the clarithromycin as compared to the combination of s1m1 and s1m2 mosaics since they are cagA positive. The presence of s2m2 strains induces gastric epithelia inflammatory which is perceived as the contributing factor to the decrease of the antibiotic delivery and can impede the H pylori eradication. Additionally, it can be noted that antibiotics activities alter the metabolic division of cells and the positive cag A strain may increase rapidly as compared to negative cagA and hence being susceptible to antibiotics (3,8).

The Antimicrobial-resistant for H. pylori has been of great concern to the medical settings due to the increased cases of resistance. A research study done by (8) aimed to evaluate the prevlance of Antimicrobial-resistant among the patients infected with H pylori. The study comprised of 1620 genotype sequences of H pylori isolations which were collected from PATRIC database. The authors highlighted the antibiotic susceptibility and the genome sequence of 10 H pylori strains that were suspected to have Antimicrobial resistance from 2017-2019 in India. The samples collected were put under the anti-microbial susceptibility and tested for afar dilutions versus 5 frequencies used in anti H pylori therapy (metronidazole, clarithromycin, furazolidone, tetracycline, and amoxicillin). As shown in the table below, out of the twelve strains, 2 strains were resistant, 4 strains were antibiotics, and 3 strains were resistant while the other 3 were antibiotics. The TMC 199 and TMC 96 strains emerged to be resistant on the

metronidazole while TMC 269 was only resistant to amoxicillin. Only one strain, TMC 125, was sensitive to all 5 antibiotics.

Genotype	No. (%) of strains			P
		Total	*cagA* positive (*n* = 44)	*cagA* negative (*n* = 74)
s1/m1 or *s1/m2*	38	35 (79.6)	3 (4.0)	*P* < 0.0001
s2/m2	72	3 (6.8)	69 (93.2)	*P* < 0.0001
s2/m1	4	3 (6.8)	1 (1.4)	NS[a]
Mix	4	3 (6.8)	1 (1.4)	NS

cagA status	No. (%) of strains				
		Total (*n* = 117)	Clarithromycin susceptible (*n* = 75)[a]	Clarithromycin resistant (*n* = 34)	Discrepant (*n* = 8)
Positive	44	36 (81.8)	5 (11.4)	3 (6.8)	
Negative	73	39 (53.4)	29 (39.7)	5 (6.8)	

Table 6: vacA genotype distribution among 118 cagA-positive and cagA-negative H. pylori strains

2.3.5. Patient-Related Factor

Though a large number of cases of primary treatment failure has to do with clarithromycin resistance secondary to gene mutation (A2143G), poor compliance to treatment medication is one of the primary factor that results in treatment failure. Perhaps, patients plays an essential role in enhancing their recovery and eradication of H. pylori and any other infections. Complying with medical prescription improve the body ability to fight against enzymes that causes the prevalence of H. pylori. Another significant patient related factor that can inhibit the eradication rate for H pylori is smoking among the patients (5). A study done by (8) showed that smoking is negatively correlated to H pylori infections. This implies that the risk and prevalence of this infection decreases linearly when patients cease smoking

cigarettes. Another significant patient related factor that influence the rate of H. pylori eradication is the number of medication doses prescribed to the patient. For instance, administering amoxicillin 1000mg twice daily is considered to more effective as compared to when it's administered once a day. However, the compliance of the patient to ensure they take the medications and prescribed by the healthcare professionals influence greatly the eradication rate.

2.3.6. Gender, Age, and Geographic Factors

Michael S. Osato, Ph.D., and colleagues in Pattern of Primary Resistance of Helicobacter pylori to Metronidazole or Clarithromycin in the United Stated, illustrated the relationship between H. pylori resistance to antibiotics (Metronidazole and clarithromycin) in regards to patient age, sex, and region of the United States. Based on the study, it was determined that women were more likely to have metronidazole resistance H.pylori than men. The rationale for having metronidazole resistance in women greater than in men is that metronidazole-containing treatment regimens are used by women to treat gynecological infections. In regards to the age of the patient, there was significant on both the metronidazole and clarithromycin resistance rates. According to the study, it illustrated that either metronidazole or clarithromycin resistance was lower in those older than 70 years than in those of middle age. Furthermore, while age and gender had significant effects on resistance rate, regional differences were not present

(13). However, other studies have found no significant correlation between ages, sex on the prevalence of clarithromycin resistance (9).

CHAPTER 3 TREATMENT OPTIONS:

Antibiotic resistance and poor patient compliance to medication are considered as significant predictors for the treatment failure of H pylori. As stated earlier, the eradication rates generated by triple therapy using clarithromycin has seen a significant decrease due to the gradual increase of clarithromycin and metronidazole resistance. Unlike clarithromycin resistance, metronidazole can be curtailed with the use of high dosage of metronidazole or either adding the PPI to the bismuth and metronidazole. On the other hand, clarithromycin resistance has a high chances of remaining the same even after of increase of the clarithromycin dosage (4). The rising rates of antibiotic resistance have largely been due the increased spread of the different antibiotics such as clarithromycin for respiratory tract infections and metronidazole for gynecological infections. This in turn has led to the increased occurrence of H pylori resistance among the patients. Though clarithromycin resistance rates vary around the world, one thing is current. That is, clarithromycin resistance in treating H Pylori is gradually increasing, and alternative primary therapies will be necessary for successful H. pylori eradication.

In the U.S, the current recommended guideline for an initial H. pylori treatment regimen is clarithromycin-based triple therapy. Clarithromycin-based triple therapy comprise of: PPI, amoxicillin, clarithromycin for 10-14 days, is recommended in a patient without penicillin (PCN) allergy, and who hasn’t yet received the macrolide. For the penicillin allergic patients and how haven’t

received macrolide initially or cannot tolerate quadruple bismuth therapy, clarithromycin is the recommended regimen which can be implemented using amoxicillin with metronidazole (4, 12, 14,16). Moreover, bismuth quadruple therapy, which comprise of PPI or H2 blocker, bismuth, metronidazole and tetracycline for 10-14 days, can also be used as a primary treatment therapy. However, the American College of Gastroenterology on the Management of H pylori infection recommends that quadruple bismuth therapy be used as reserved for individuals who have failed one course of H. pylori treatment or who have PCN allergies. Clarithromycin-based triple therapy's eradication rate is between 70 to 85 percent. Whereas the bismuth quadruple therapy’s eradication rate is between 75 to 90 percent (4, 14).

Various Italian studies has shown that eradication rates for H pylori is above 90% when using the sequential therapy accompanied by PPI and amoxicillin for the first five days. The use of clarithromycin plays an essential role in reducing the eradication rate and prevalence of H. pylori among the patients with this infection. This can be enhanced by the use of clarithromycin-based triple therapy. Additionally, sequential therapy is perceived to be more superior as compared to clarithromycin-based triple therapy among the patients who are experiencing the H pylori strain (4). Another multicenter trial investigated the effectiveness of sequential therapy and found that it was 85% effective as compared to clarithromycin triple therapy that showed an effectiveness of 44% among the

patients infected with H pylori. Furthermore, sequential therapy had a compliance of over 90%, and showcased side effects that were not prevalent as compared to those of clarithromycin triple therapy. Details regarding the dosage of sequential therapy, duration of therapy, and eradication rate can be seen in Table 1.

For the patients who have a persistent infection after failing clarithromycin triple therapy, bismuth-based quadruple therapy is considered as the alternative therapy which can be administered within 7-14 days with a 80% eradication rate when applied as a second line therapy (4,8). Various scholars and researches has tried to identify an alternative for the bismuth-based quadruple therapy. For instance, one of the Australian based research evaluated 140 patients who had failed, clarithromycin, and amoxicillin PPI therapies and were treated with alternative antibiotics such as 150mg rifabutin and 80 mg Pantoprazole. The study concluded that using these alternative antibiotics, the eradication rate was 91% while the clarithromycin or metronidazole resistance has little or no influence on the treatment procedure. However, Rifabutin, has serious rare side effects such as myelotoxicity, ocular toxicity, and red discoloration of urine (4).

Levofloxacin is a fluoroquinolone antibiotic, and the adoption of Levofloxacin-based triple therapy (LBTT) has been considered for a possible alternative to clarithromycin triple and bismuth-based quadruple therapies. Currently, Levofloxacin-based triple therapy is regarded as 2rd and 3rd line therapy among the patients with persistent H. pylori infections. Recent studies has shown

that 10-day regimen of the LBTT results to high eradication rate as compared to the 7 day bismuth-based quadruple therapy course. The eradication rate for these two methods is 87% and 68% respectively (4). Eradication rate for levofloxacin-based triple therapy and bismuth based quadruple therapy were 87% and 68%, respectively (4). Details regarding Levofloxacin triple therapy can be seen in Table 7 (4).

Regimen	Duration	Eradication Rates	Comments
Bismuth quadruple therapy PPI q.d. tetracycline, Pepto Bismol, metronidazole q.i.d.	7	68% (95% CI 62–74%)	Accessible, cheap but high pill count and frequent mild side effects
Levofloxacin triple therapy PPI, amoxicillin 1 g b.i.d., levofloxacin 500 mg q.d.	10	87% (95% CI 82–92%)	Requires validation in North America

Table 7: Salvage Therapies for Persistent H. pylori infection

Probiotics:

A research done by (5) showed that application of probiotics to manage H. pylori is not certain and comprise of various controversies due to the inconsistencies on its formulation, dosage, timing and duration of therapy. The Toronto consensus as differed with the AGG about the use of probiotics with the former argues that probiotics is not effective for patients use while AGG advocating for its use. Some literatures and clinical trials has suggested that addition of probiotics such as Saccharomyces boulardii and Lactobacillus species to the regimens increases the eradication rate by 9% and5% respectively while also decreasing the side effects such as diarrhea by 14% and 7% respectively (5).

CHAPTER 4

DISCUSSION

Current recommended guidelines for initial H. pylori treatment regimen in the United States is clarithromycin-based triple therapy, and if the clarithromycin-based triple therapy fails or if the patient has PCN allergy, then bismuth-based quadruple therapy has been recommended as the rescue therapy for H. pylori eradication (4,14,16). However, the efficacy of clarithromycin-based triple therapy has decreased resulting in an increased rate of treatment failure. During the last two decades, widespread use of current antibiotics (i.e. clarithromycin for respiratory infection) in the general population has increased the occurrence of primary H. pylori resistance. Due to widespread use of clarithromycin, H. pylori resistance strain has occurred. H. Pylori resistance to clarithromycin correlates with a point mutation in the 23S rRNA gene. The most common point mutation is A21343G, which is largely responsible for clarithromycin resistance in H. pylori strain (1, 3, 7, 9, 10). Once the resistance to clarithromycin has occurred, increasing the dose of clarithromycin could not overcome the resistance; thus, in patients with primary treatment failure, every effort should be made to avoid antibiotics that have been previously taken by the patient. Whereas, in inpatient patients with metronidazole resistance, to some extend, metronidazole resistance can be overcome by using higher doses of metronidazole and the addition of a PPI to bismuth, tetracycline, and metronidazole (4).

Patients with treatment failure, the clinician should prompt endoscopy, culture and susceptibility testing. Retreatment should exclude antibiotics with acquired resistance. However, at present no gold standard method has been proposed for testing H. pylori susceptibility to antibiotics. There is still a need for standardization regarding the appropriate medium, the supplementation, and the size of the inoculums, the incubation atmosphere, and the appropriate time to read the plates and the breakpoint differentiating resistance and susceptibility (15). Since cross-resistance exists for macrolide, erythromycin susceptibility testing may be useful in determining clarithromycin resistant H. pylori strains. Furthermore, due to the association between point mutation on 23S rRNA and macrolide resistance, another possible approach to determine clarithromycin resistance in the H.pylori strain is using polymerase chain reaction (PCR) based molecular technique. PCR-based techniques include polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP), an oligonucleotide ligation assay (PCR-OLA), a DNA enzyme immunoassay (PCR-DEIA), a reverse hybridization line probe assay (PCR-LiPA), and preferential homoduplex formation assay (PCR-PHFA). The uses of PCR-based molecular techniques are quicker than microbiological susceptibility testing, and more importantly, they can be performed directly on gastric biopsies and gastric juice (10,15). Though culture or PCR are the primary means by which antibiotic sensitivities can be determined, neither is widely available for clinical use in the United States. Thus

currently no guidelines have been placed, in which it would implement clinicians to use PCR based technique to determine clarithromycin resistance H.pylori strain individuals with primary treatment failure.

In addition, due to increased clarithromycin resistance and decreasing eradication yield with clarithromycin based triple therapy, alternative treatment regimens need to be considered. Sequential therapy, which consists of PPI and amoxicillin for 5 days followed by PPI, clarithromycin, and tinidazole for an additional 5 days has shown to achieve superior to clarithromycin-based triple therapy and better patient compliance rate. Levofloxacin-based triple therapy (PPI, Levofloxacin, and amoxicillin for 10 days) has shown to be more effective and better tolerated than the current bismuth quadruple therapy in patients with persistent H. pylori infection. These two treatment regimens have shown promising alternative treatment in providing successful eradication of H. pylori (4). However, these regiments need to be further studied in the United States prior to getting validation. In conclusion, until newer treatment such as sequential therapy or levofloxacin-based triple therapy becomes available and validated in the United States, the use of PPI, clarithromycin, and amoxicillin or metronidazole for 10-14 days remain the first-line treatment regimen for H. pylori eradication. In addition, in individuals with PCN allergy or failed with clarithromycin based treatment, then bismuth-based quadruple therapy for 7-14 days is acceptable either as first-line or second-line therapy.

Impact of Clarithromycin Resistance on H. pylori Eradication

Research done by (7) aimed to evaluate the impact of clarithromycin resistance on the process of eradicating prevalence of H pylori among the infected adults. The study showed that the eradication of H. pylori occurred in 80% among the patients experiencing clarithromycin-sensitive isolated as compared to patients with clarithromycin-resistant, as shown in the table below.

Isolate[a] (no.)	No. of patients[b] (%)	No. of isolates with eradication failure (%)	MIC (µg/ml)[b]	
			Amoxicillin	Clarithromycin
Wild type (91)	91 (79.83)	0 (0.00)	0.016-0.063 (S)	0.031-0.125 (S)
A2142G (20)	20 (17.54)	20[c] (17.54)	0.031-0.125 (S)	32->256 (R)
A2143G (3)	3 (2.63)	3[c] (2.63)	0.016-0.125 (S)	4-128 (R)

Table 8: A-G mutations of Clarithromycin resistance H. pylori isolates

Based on the above table, it was noted that the failure rate for bacterial eradication in A2142G and A2143G mutation group was similar, while the failure rate for bacteria eradication correlated with two mutations. The study also showed that there exists a huge difference in the rate of bacteria eradication among the H pylori patients with clarithromycin-resistance and clarithromycin-sensitivity isolates. This implies that the clarithromycin resistance incidence is relatively higher in many cases across the globe.

This shows that Clarithromycin resistance is considered to be relatively 100% predictive to the treatment failure and hence it reduces effectiveness by approximately 60%. These differences in rates is due to the variation in

breakpoints which are used and the various mutations involved in resistance mechanisms. However, increased level of resistance can't be countered by increasing doses or therapy duration. With this concept, it can be noted that endoscopy, culture and susceptibility testing should be evaluated effectively during the clinical experiments to enhance treatment success and avoid treatment failure that can result from these factors.

The essential risk factor for clarithromycin resistance is considered to be the consumption of macrolides. This resistance is high in children since there is the increased prescription of drugs as compared to that of adults. Perhaps, in children, respiratory tract infections have been common in the past decade, which has increased drug prescription among children across the world. For instance, in Japanese families, it can be noted that even though children strain and that of their parents is identical/similar, the children strain is likely to become clarithromycin resistant after they are treated for clarithromycin. However, there was no relationship with age noted among the adults in many studies and clinical observations, which shows that the clarithromycin resistance is more prevalent in children than in adults.

Based on the literature review, various authors compared the consumption of macrolide and the resistance in different countries over a given period. Even though various studies have recognized that macrolides comprise of vitro cross-resistance, none of the researchers has shown that if all the macrolides has similar

potentiality of selecting the strain in vivo based on the gastric mucosa diffusion. Notably, according to the vitro data, when gastric mucosa contain clarithromycin at the sub-inhibitory concentrations, the strains are detected and selected within the human body. However, for the macrolides that are not ready for such sub-inhibitory concentrations, there is little or no impact on the process of selecting the clarithromycin resistance. Additionally, when there is a treatment failure, the emergent resistance is likely to take place in at least two thirds of the strains. In many countries, the average rate of secondary resistance is considered to be 25% after azithromycin therapy regardless of the high failure rate. Therefore, it can be noted that the impact of azithromycin on the resistance strain selection is of great importance when determining the clarithromycin resistance.

During clinical trials, the clarithromycin resistance is likely to face various controversies that may lead to mixed results. One of these controversies is the mutation stability which leads to the resistance. Various studies showed that there were few positions particularly in the 23S rDNA, in which mutations were found and spatial configuration can change to ribosome. However, it can be understood that there is huge biological cost related to mutation and hence cannot be managed effectively in case of stoppage of the selection pressure. In many cases, resistance mutants are related to the vitro subculture and at different vivo time-points, the resistance is likely to remain and hence showing clarithromycin prevalence is likely to be high when the mutation resistance in human bodies is high.

It is prudent to note that mutations involved in clarithromycin resistance is one of the significant aspects of consideration. Perhaps, PCR-RFLP is used to determine the point mutations embedded on the clarithromycin resistance where the most frequent mutation is considered to be A2143G (68.9%) but the prevalence of these mutations varies from 55% to 93% and A2142G (11.5%) and A2142C (2.8%).

Detection of clarithromycin resistance is one of the significant aspects that should be considered when evaluating the eradication of H. Pylori. Various methods are used to determine the clarithromycin resistance as shown in the table below (8)

Based on amplification of 23S rRNA gene
- Sequencing
- RFLP (restriction fragment length polymorphism)[131,132]
- OLA (oligonucleotide ligation assay)[133]
- DEIA (DNA enzyme immunoassay)[134,135]
- PHFA (preferential homoduplex formation assay)[136]
- INNO-LiPA (line probe assay)[137]
- Mismatch PCR[138]
- DG-DGGE (double gradient-denaturing gradient gel electrophoresis)[139]
- FRET (fluorescence resonance energy transfer)[140–144]
Based on hybridisation
- FISH assay (fluorescence in situ hybridisation assay)[145]

Table 9: Molecular methods for H pylori testing of clarithromycin resistance

Based on the above molecular methods of H. Pylori testing, real time PCR is the most significant approach that can be applied in the near future. Perhaps, the

Lightcycler is tailored to perform quantitative PCR and ascertain the severity of the clarithromycin resistance and its ability to eradicate the H. Pylori from the human body. The fluorescence resonance energy transfer (FRET) has emerged as one of the most promising technology that is used to screen the clarithromycin mutations and ascertain the H. pylori prevalence and the best and appropriate method of its eradication this was first tested and applied in 2009 where the results showed a double DNA strand with a specific fluorophore SYBR Green 1 on the probe which were used to test the strains in H. Pylori. During this process, it can be noted that in case of anchor probe exit, energy can be transferred to the sensor probe which in turn emits the H. Pylori signals. After the amplification process, the next step is conducting melting step that results to various melting points wild type mutations. The importance of this method is that it helps to detect H. Pylori and it's sued to determine the clarithromycin susceptibility and how it's related to the biopsy specimen. Therefore, this process is of great importance in showcasing the presence of clarithromycin resistance in H. pylori.

Another method of detecting the clarithromycin resistance is fluorescence in situ hybridization (FISH). This is a method which is used to detect and locate where the sequential DNA chromosomes are located. This method also involves exposing chromosomes to tiny DNA sequences known as probe that comprise of fluorescent molecules. The FISH method is applied in detecting the presence of H pylori and the clarithromycin resistance and also ascertain its level and prevalence.

This is because the FISH method comprises of rRNA hybridization which is detected by fluorescent labelled oligonucleotide probes. This method is important as it allows the clinicians to detect the presence of H. Pylori with a 14S rRNA. Another importance of this method is that it provides the clinical officers and researchers with a way to visualize and map the genetic materials in the patient's cells which includes specific genes and gene portions. This in turn can be used to understand the variety of chromosomal abnormalities as well as other genetic mutations found in H. pylori.

Based on these methods, it can be noted that there is variations on infections between clarithromycin susceptibility and the frequency of bacteria resistance. Various laboratory studies have shown that variable genotypes are experienced in 25% of cases. Notably, the significant aspect of the population of bacteria conforms to the genotype susceptibility which in turn indicates that clarithromycin resistance occurs impulsively and it has a tendency of remaining at a low level and it's subsequently used by the drug administration agencies.

The H. Pylori resistance to the clarithromycin is perceived to correlate significantly with A2142G and A2143G transition mutation in the 23S rDNA which in turn results in a reduction of affinity of the clarithromycin that binds ribosomes together. The clarithromycin resistance prevalence in children and adults reflects the frequency of prescription of macrolides for treating RTIs apart from H. pylori. Various researchers have shown that there is a high dominance of

A2143G mutation in resistant isolates as compared to the A2142G resistance isolates. This implies that H. pylori comprise of high degree of genetic variability, different mutations/genes of H. pylori can differ from different countries and locations.

Various studies has shown that Clarithromycin resistance is 100% predictive for the treatment failure while on other studies, Clarithromycin resistance reduces treatment effectiveness by 60%. The difference between these eradication rates can be understood by different breakpoints which are applied in various mutations in the resistance mechanism. However, high resistance level can't be solved by increasing the therapy duration or dosage. It is prudent to note that treatment failure leads to endoscopy, culture and susceptibility testing. On the other hand, retreatment should not include antibiotics with acquired resistance. Various researches have indicated that retreatment difficulties can be solved through first line treatment regimen, which helps to increase the H. pylori eradication rate. It can also be noted H. pylori eradication rate can also be improved by performing antibiotic therapy which results to anti-microbial susceptibility.

Therefore, based on the above discussion, it can be noted that different approaches and antibiotics can be employed in the pursuit of eradicating Clarithromycin resistance among the patients with H. pylori infections. However, in many cases these results doesn't base on the pathogenic differences but rather they are based on the protagonist expression of the isolated groups. This study also

noted that H. pylori anti-microbial susceptibility testing can be used prior to selecting the first triple-drug therapy among the patients with H pylori. Also, clarithromycin cannot be effective in treating primary resistance cases.

Risk Factors Associated with Clarithromycin Resistance

It is prudent to note that pretreatment for the anti-microbial resistance of H. pylori has a negative influence on the treatment effectiveness among the patients suffering from H. pylori. Notably, clarithromycin is one of the most comprising risk factor that inhibits the eradication of H. pylori. The anti-microbial resistance has a great impact of reducing the treatment effectiveness by 55%. However, not all regimens are equally affected by this risk factor, but it depends on the immune system of the individual suffering from H. pylori. Table 2 above shows that anti-microbial resistance has a great effect on the dual therapy when clarithromycin is applied together with ant-secretory agents. In this case, the dual therapy is likely to fail by 95% among the resistance pretreatment isolates. It is also prudent to note that even though triple-drug therapy is less affected by this process, it has a 68% tendency of failure among the resistant pretreatment patients.

It was also found that pretreatment resistance doesn’t all the time predict the reduction of treatment and clinical effectiveness. The risk factor that inhibit eradication of H. pylori in this case is lack of standard vitro approach that can help to determine susceptibility. However, (9) showed that metronidazole comprising of regimen resistance reduces clinical effectiveness by 38%. On the other hand, (10)

the findings showed that nitroimidazole comprising triple therapy regimen attained 92% eradication of resistant strained among the patients with H. pylori. Another significant aspect that emerged during the study about clinical effectiveness on eradication of H. pylori is amoxicillin resistance. Although this occurs rarely, it plays a great role in reducing clinical effectiveness of dual therapy when using amoxicillin. However, the clinical effectiveness of amoxicillin resistance in the triple therapy is not yet known as there are no studies or clinical trials that has researched about it.

Another significant risk factor that is associated with clarithromycin resistance is geographic location. For instance, a study showed that the resistance rates were high in the mid-Atlantic region as compared to northern Atlantic region. However, the research about the reason why clarithromycin resistance differs with different geological locations has not been done. Therefore, prescribing drugs using oral macrolides is likely to have a great effect, but data utilization about macrolide has not been known in any given geographical location.

Age is another is another significant factor that is associated with clarithromycin resistance. Perhaps, older people have higher chances of experiencing clarithromycin resistance while young aged people are associated with metronidazole resistance. In the same sense, it can be noted that elderly patients have higher chances of getting respiratory tract infections where macrolides are prescribed while the young females who are suffering from

gynecologic infections are treated using metronidazole. For young females aged 20-40 years, metronidazole resistance is considered to be common, especially in European countries. In many studies, it has been noted that females significantly related to resistance for both clarithromycin and metronidazole and particularly who are aged 40 years and below. However, it is not yet known the reason why females are more likely to have higher clarithromycin resistance than men.

The last risk factor that is related to clarithromycin resistance among the patients with H. pylori is ethnicity. This study noted that white people have a relatively low prevalence of metronidazole resistance than the people of color and especially black people. For instance, Asian people has a high chance of metronidazole resistance. However, the reason of high clarithromycin resistance among the white people is not yet known but the clinical observations has stated that white people are likely to have higher tendency of clarithromycin resistance and hence slow eradication of H. pylori among the patients.

patients with inactive ulcers are likely to have a high chance of having clarithromycin resistance as compared to the patients with active ulcers. This is because patients with inactive ulcers are in many cases exposed to unsuccessful therapies related to the eradication of H. pylori. This in turn explains the reasons for high rates of clarithromycin resistance among this population. However, due to the increased cases of clarithromycin resistance among people with inactive underlying comorbidities, clinical practitioners should inquire from the patients

about the previous use of anti-microbial and particularly macrolides before choosing the best and appropriate treatment regimen.

Indications for *H. pylori* Testing and Treatment

H. pylori testing is indicated in different patients with underlying comorbidities related to this infection. Perhaps, patients who show positive results for H pylori are required to seek an immediate medication to prevent the infection from getting worse. For instance, patients with inactive or previous peptic ulcers has high chances of testing positive for H. pylori uncles the patients shows the documents and records shown that the infection was cured. Additionally, other patients who should be tested include those with low grade gastric mucosa and those with past history of endoscopic resections. However, the use of gastro esophageal reflux disease to test patients is not recommendable unless the patient peptic ulcer history or any other underlying illness related to peptic ulcer. This implies that of the patient experiencing gastro esophageal reflux disease test positive for H. pylori, clinical practitioners should offer treatment by acknowledging signs and symptoms shown by the patient.

The American College of Gastroenterology recommends that H. pylori testing involves identifying active infection which includes urea breath tests, fecal antigen tests as well as endoscopic biopsy testing. Due to the higher probability of infection among the patients with recorded peptic ulcer diseases, H. pylori testing using immunoglobulin G antibody is accepted among the patients with peptic

ulcers. On the other hand, for the patients over 60 years of age, can be tested using nonendoscopy which investigates red flags in dyspepsia. Notably, there is little evidence and recommendations about treatment and testing for the asymptomatic patients which has a family history related to gastric cancer peptic ulcers. The clinical research has shown that H. pylori can be treated by combining various antibiotics and PPI. Clinicians should also ask patients about their previous exposure to antibiotic, which in turn can help to guide the treatment regimen. However, it should be noted that there is no 100% cure rate for H. pylori infections.

CONCLUSION:

1. Helicobacter pylori (H. pylori) has remained one of the main challenges in the medical setting due to the widespread infections across the world wrong the fact that it is highly contagious and can be spread through physical body contact. H pylori is associated with various gastrointestinal infections such as chronic gastritis, GERD, functional dyspepsia, peptic ulcer infections, gastric cancer, and MALT lymphomas. H. pylori infection causes duodenal ulcers and gastric ulcers. Providing successful treatment not only successful eradicates the infection but it also improves symptoms, and decreases the rate of recurrences of either duodenal or gastric ulcer.

2. In the United States, the current first line treatment for eradicating H. pylori comprise of various antibiotic procedures such as PPI, amoxicillin and clarithromycin for duration of 10-14 days, inpatient without PCN allergy and inpatient with PCN allergy it has been recommended that amoxicillin should be substituted with metronidazole. Perhaps, bismuth-based quadruple therapy is used as an alternative in case of failure of clarithromycin therapy. In addition, bismuth-based quadruple therapy can also be used as a primary treatment regimen in patients with PCN allergy or if the patient is known to have clarithromycin resistance. Different researches has indicated that H. pylori antibiotic resistance has gradually increased around the world. However, the rate of antibiotic resistance and the type of antibiotic resistances vary from country to country. In addition,

American has the highest clarithromycin resistance, followed by Asia and then Europe. The study also illustrated that Africa has among the highest Metronidazole resistance, followed by America, then Asia and finally Europe. For the European nations, Spain reported the highest rate of clarithromycin resistance and the lowest clarithromycin resistance among the European countries were Sweden and the Netherlands respectively.

3. The two most important factors in predicting treatment failure were antibiotic resistance and poor complaints. It has been implicated that clarithromycin resistance was due to point mutation in the peptidyltransferase domain V of the 23S rRNA. From all the subsets of point mutation, three most common point mutations were A2143G, A2142G and A2142C, and A2143G being the most common point mutation that is primarily responsible for clarithromycin resistance in H. pylori Strain. The rising rate of clarithromycin resistance in the H. pylori strain is largely due to widespread use of clarithromycin for treatment other than H. pylori infection (i.e clarithromycin for respiratory infections) in the general population. In addition, H. pylori eradication rate has also decreased due to Metronidazole resistance as well. It has been determined that metronidazole resistance in women is greater than men. Like clarithromycin resistance, metronidazole resistance has also occurred largely due it widespread use other than H. pylori infection treatment (i.e metronidazole for gynecological infections, parasitic infection, and sexually transmitted disease). Besides the antibiotic

resistance, poor compliance was also an issue in regard to treatment failure. A Study on Failure of Helicobacter pylori eradication in an ambulatory population conducted by Wermeille J, Deterding JP and Cunningham M, illustrated that patient compliance played an important role in successful eradication. However, a significant proportion of the treatment failure could not be explained by this study and thus alternative factors such as antibiotic resistance needed to be taken into consideration.

4. In the United States, the current recommended guidelines for initial H. pylori treatment regimen is clarithromycin-based triple therapy. If clarithromycin-based triple therapy fails or a patient has PCN allergy, then bismuth-based quadruple therapy has been recommended. However, due to clarithromycin resistance and decreased eradication yield with clarithromycin-based triple therapy, several studies have looked at different antibiotics in order to find effective treatment regimens, with good patient compliance with minimal antibiotic side effects. Two possible treatment regimens shows to be promising, but need further studies in the United States prior to getting validated in North America. The first treatment regimen was composed of PPI and amoxicillin for 5 days followed by PPI, clarithromycin and tinidazole for an additional 5 days. This regimen achieved eradication rates superior to clarithromycin-based triple therapy and had a better patient compliance rate. The second regimen was levofloxacin-based triple therapy (PPI, levofloxacin and amoxicillin for 10 days) showed to be more effective and

better tolerated than bismuth quadruple therapy in patients with persistent H. pylori infection. But until the two regimens get validated, clarithromycin-based triple therapy remains the first-line regimen for H. pylori eradication.

5. Also, individual with persistent H. pylori with treatment failure from first-line treatment regimen or second-line treatment regimen, the clinician should prompt the patient to have repeat endoscopy with culture and susceptibility testing done to determine which antibiotic the patient is resistant to and to which antibiotic the patient is susceptible too. The H. pylori eradication rate had significantly improved when antibiotic therapy was given on the basis of the results of an anti-microbial susceptibility test. However, currently no gold standard method has been proposed for testing H. pylori susceptibility to antibiotics. Though culture or PCR are the primary means by which sensitivity can be determined, neither of the tests are widely available for clinical use in the United States. Thus currently, no guidelines have been placed, in which it would implement clinicians to use PCR based techniques to determine clarithromycin resistance H. pylori strain individuals with treatment failure.

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