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Helicobacter Pylori Infection in Humans: Emerging Resistance Patterns, Diagnostic Challenges, and Novel Therapeutic Strategies

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Annotation: Helicobacter pylori (H. pylori) is a microaerophilic, spiral-shaped, gram-negative bacterium that primarily colonizes the gastric epithelium and is opportunistically responsible for chronic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma, and gastric cancer. The human gut plays host to a broad-spectrum microbiome that is considered to be a critical factor in maintaining gut health and stability. However, H. pylori is one of the few bacterial species that can colonize the acid-secreting stomach, where it faced highly acidic conditions in the gastrointestinal tract. H. pylori infection is one of the most prevalent global chronic bacterial infections and is classified as a group I carcinogen. The overall prevalence of H. pylori infection is

extremely high globally, posing a global health threat.

H. pylori has been prioritized as a priority pathogen due to the increasing prevalence and the growing burden of antibiotic resistance. There is a critical shortage of treatment options for H. pylori infections that are not adequately addressed in the immediate to medium-term. New antibiotics with novel mechanisms of action are urgently needed to meet this challenge. H. pylori was discovered in 1982, and a rapid change has occurred in a remarkably short time. Since the introduction of combination therapies consisting of at least one antimicrobial agent and a proton pump inhibitor, H. pylori infection has been effectively treated for over 30 years. The development of antibiotic resistance patterns and shifts in sensitivity to first-line antibiotics are becoming a major concern in H. pylori eradication. The concomitant use of multiple antibiotics contributes to the emergent resistance patterns.

Antibiotic resistance patterns in H. pylori are evolving. In this review, the current treatment options in eradicating antibiotic-resistant H. pylori are discussed. In addition, future perspectives such as diagnostic challenges and therapeutic strategies to combat antibiotic-resistant H. pylori are emphasized. H. pylori colonizes almost 50% of the world population and is the most common chronic infection in humans. Infection with H. pylori results in both acute and chronic gastric inflammation and a marked increase in the risk of developing severe gastric diseases including peptic ulcers, gastric cancer, gastric mucosa-

associated lymphoid tissue lymphoma, and atrophic gastritis. H. pylori primarily colonizes the gastric epithelium and remains one of the only known bacterial species that live in the acidic compartment of the mammalian and avian stomach. It induces chronic gastric inflammation in almost all infected individuals, and 10% will develop severe gastric diseases.

1. Introduction

Helicobacter pylori (H. pylori) is a bacterium that infects the stomach and is associated with gastrointestinal illnesses such as peptic ulcers and gastric cancer. H. pylori has been classified as a global priority pathogen due to increasing prevalence and antibiotic resistance. H. pylori causes infections in the stomach, resulting in chronic gastritis (99.9% of cases), which can lead to peptic ulcer disease (10–15% of cases), mucosa-associated lymphoid tissue lymphoma (MALT lymphoma, 1 in 100,000), as well as gastric adenocarcinoma (1 in 1000). H. pylori is the key infectious agent for the aforementioned diseases and is linked to auto-immune diseases. Infection with H. pylori is one of the most prevalent chronic infections worldwide, with about half of the world's population infected pre-COVID-19. An estimated 10%–20% of individuals are infected in developed countries, but this prevalence increases to greater than 50% in developing countries. H. pylori infection is usually acquired in youth through fecal-oral or oral-oral transmission. The increasing incidence of antibiotic-resistant strains necessitated a paradigm shift in understanding H. pylori, its epidemiology, resistance mechanisms, and novel therapeutic strategies.

In nearly every country tested, H. pylori showed high susceptibility rates (>90%) to bismuth compounds, amoxicillin, and metronidazole. Presently, amoxicillin with any of the various proton-pump inhibitors (PPIs) remains the first line in the USA. While PPIs are a key component of most regimens, pharmacogenomic specificity is now required for optimal acid suppression. Resistance against other antibiotics is increasing with wide intercountry variability (metronidazole 60%-95%, clarithromycin 10%-45%, levofloxacin 0%-20% in developed nations, and fluoroquinolones <25%). In the USA, resistance rates to meta doxycycline, rifabutin, and bismuth compounds are very low. But the only novel antibiotic under human trials, nitazoxanide showed <50% efficacy. Consequently, challenges imposed by resistant strains and concerns regarding inadequate antibiotic discovery have prompted the evaluation of alternative strategies for H. pylori eradication.

The decline in effective therapies for H. pylori infection is alarming. Failing to eradicate resistant strains could allow H. pylori to adapt further, potentially generating more virulent or multi-drug resistant strains. Multidrug resistant strains with simultaneous resistance/elevation of MIC >256-fold against amoxicillin/clarithromycin/vancomycin were shown to amplify virulence in vivo. The failure to eradicate strains with higher initial densities and greater than 75% mutation frequency could allow H. pylori to persist and resist novel antibiotics and therapies targeting virulence. For over 30 years, H. pylori treatment has had notable clinical efficacy and relatively few side effects. The discovery of these types of therapies has also highlighted areas for further investigation in the quest to better treat H. pylori and avoid the development of antibiotic resistance. Novel subjects include: floating metallic micro-robots for in vivo monitoring and drug delivery; confinement of H. pylori bacteria with low-density polyethylene to create nano-biosensors; biomaterial assembly

of immunoglobulin–gold nanoparticles; and varied groups of RNA-targeted approaches. [1][2][3]

2. Overview of Helicobacter pylori

Helicobacter pylori (H. pylori) is a Gram-negative microaerophilic bacterium that colonizes the gastric epithelium of humans. It is a spiral-shaped organism and was first discovered in the early 1980s. Helicobacter pylori infection is one of the most common chronic infections in humans, with an estimated prevalence in the developing countries of around 50% and 10-20% in developed countries [4]. Eradication of H. pylori is associated with improvement in 50-90% of cases of dyspepsia, reduction in the recurrence of peptic ulcers and gastric cancer, improvement of mucosa-associated lymphoid tissue lymphoma, and reduction in iron deficiency anemia due to chronic inflammation. H. pylori has been classified as a group 1 carcinogen by the World Health Organization (WHO), as it is responsible for approximately 90% of noncardia gastric adenocarcinomas. In the past few decades, there have been increasing concerns on H. pylori infection due to increasing prevalence of H. pylori infection, increasing age and extent of infection in population, increasing prevalence of antibiotic resistance, and changing epidemiology of gastric malignancies.

H. pylori infection is diagnosed using invasive or non-invasive methods [5]. Invasive diagnosis can be achieved using endoscopic biopsy method. Detection of urease activity using urease test is the standard diagnostic tool for H. pylori infection. The rapid urease tests are simple to perform, inexpensive, and widely available in clinics and hospitals. However, rapid urease tests have low sensitivity for patients undergoing therapy against H. pylori. Additionally, svideo-endoscopy and performing biopsy are necessary to diagnose H. pylori, which is the limitation of the endoscopic biopsy method. To overcome this limitation, non-invasive diagnostic tests have been developed to detect H. pylori infection without the need of endoscopy. Among these tests, the UBT is the most accurate non-invasive technique in terms of sensitivity and specificity. H. pylori infection is confirmed by C13-urea breath test by performing spectrophotometric measurement of 13CO2. However, these non-invasive tests also have important limitations. No one test is 100% sensitive and specific for diagnosing H. pylori infection. Therefore, combination tests should be performed to reduce the false-negative and false positive results.

3. Epidemiology of Helicobacter pylori Infection

Helicobacter pylori is a major global health problem affecting around half of the world's population [6]. The prevalence of H. pylori infection varies markedly according to geographical region with the highest rates in developing countries. H. pylori is either acquired in infancy and carried lifelong, or acquired later with increased risk at lower socioeconomic status. It is generally believed that H. pylori is predominantly acquired by oral rather than oral-fecal transmission. Studies have detected seroconversion of H. pylori antibodies coinciding with infectious diarrheal disease and also detected the bacterium in fecal samples. However, after the onset of H. pylori detected in the gastric mucosa, subsequent infestation was not seen in feces, arguing against fecal-oral transmission in later life. Water, food, as well as mother-child transmission has all been suggested to play a role. H. pylori infection is responsible for serious diseases of the upper gastrointestinal tract, including peptic ulcer disease and gastric adenocarcinoma [7]. To treat H. pylori infections, various treatment regimens have been proposed and employed in clinical practice. However, antibiotic resistance has been increasing worldwide and has become critically important in the treatment of H. pylori infection for the public health sector. The World Health Organization has listed H. pylori as a high-priority pathogen. In high-income countries, the implementation of some public health policies has improved hygiene and socioeconomic status. Consequently, the incidence and prevalence of peptic ulcer disease caused by H. pylori infection have declined markedly, along with the eradication rates. Nevertheless, in developing countries, the prevalence of prevalent H. pylori infection and disease persists high. Improvements in hygiene and living conditions are important factors in decreasing the prevalence of infection.

4. Pathogenesis of Helicobacter pylori

Helicobacter pylori has attracted attention since its first discovery in 1983 due to its unique characteristics. H. pylori is a spiral-shaped, flagellated Gram-negative bacterium, which is the only bacterial species known to invade the stomach. It is also the first organism found to be classified as a Class I carcinogen by the WHO. Prevalent in humans, H. pylori infection persists throughout life unless specifically treated, resulting in chronic inflammation of the gastric mucosa. The persistence of H. pylori to be one of the most prevalent human pathogenic microbes worldwide is mostly due to its complex strategies for modifying and adapting to the hostile environment of the stomach. Infection by H. pylori can lead to gastric cancer, ulcers, and gastritis, posing a public health risk and economic burden to the global community [8].

H. pylori usually occupies the mucus layer adjacent to epithelial gastric cells. A variety of mucous glycoproteins such as MUC5AC and MUC6 were shown to be its receptors. H. pylori uses its natural motility through flagella to penetrate into the mucous layer, which protects itself from the gastric acid. Optimization of the swimming response to pH alterations and chemokinesis toward negative soluble nitrate gradients may help H. pylori maintain its position and motility in the gastric milieu. As a fastidious bacterium, H. pylori can also withstand a low pH environment by producing a urease-like enzyme to produce alkaline ammonia from the catalysis of urea, in order to counteract gastric acid secretion. In addition to neutralizing acid, the urease from H. pylori is involved in colonization as it enhances the mucous adhering ability and plays a role in chemotaxis.

The urease-defective mutant is less virulent for Mongolian gerbil models than the wild-type strain. Colonization is much lower in the mutant-infected conditions, suggesting that urease is required for H. pylori colonization and pathogenesis. H. pylori can modulate host immunity by shedding microfilaments from the outer membrane in order to modify the mucosal immune response. Host Phospholipase inhibitors have been reported to inhibit OMV shedding, resulting in reduced Jun-N-terminal Kinase activation.

5. Clinical Manifestations

H. pylori infection can be asymptomatic but is associated with chronic gastritis and numerous complications. The common complications of H. pylori infection include functional dyspepsia, peptic ulcer disease, stomach cancer, and extra-gastric disorders. H. pylori infection is considered a group 1 carcinogen by IARC, and the International Consensus Gastric Cancer Group recommends eradication as a preventive measure against gastric cancer. H. pylori gastritis is categorized as an infectious disease under aetiology and pathological classification [5].

Functional dyspepsia (FD) is a common disorder characterized by troublesome symptoms originating from the gastroduodenal region that do not warrant an organic explanation. There are two main clinical subtypes of FD: postprandial distress syndrome and epigastric pain syndrome. The cornerstone of treatment for FD is antisecretory acid-suppression therapy, which provides symptom relief in many patients. However, some patients remain symptomatic despite receiving appropriate conventional therapy. Furthermore, it is challenging to make a proper diagnosis when patients present with dyspeptic symptoms. Recent advances in gastrointestinal (GI) endoscopy techniques have enabled the advent of device-assisted enteroscopy, which allows across-the-board checking of the entire small bowel [7].

Peptic ulcer disease is one of the complications of H. pylori infection. Peptic ulcers include chronic, solitary lesions of the gastrointestinal tract, generally of more than 5 mm diameter, which can be found in the gastric (gastric ulcer) or duodenal (duodenal ulcer) region. An estimated 5%-10% of individuals with H. pylori infection will develop a peptic ulcer disease in their lifetime. Peptic ulcers can lead to bleeding, perforation, and obstruction. H. pylori are present in over 90% of patients with duodenal ulcers. In gastric ulcers, the incidence is also high, approaching up to 85%. H. pylori eradication is an important part of treating peptic ulcers. H. pylori accounts for the majority of peptic ulcers; therefore, once H. pylori is eradicated, the ulcer will usually heal, and

symptoms will disappear.

6. Current Diagnostic Methods

The presence of Helicobacter pylori may be demonstrated with a variety of laboratory techniques designed to identify produced urease, biochemical characteristics, and nucleic acid detection. Non-invasive and invasive tests can be used for H. pylori diagnosis. The invasive tests require an endoscopic procedure with gastric mucosa sample collection and include histological examination, culture, and urease tests [9]. Non-invasive tests use chemical compounds that indicate the presence of H. pylori, such as urease activity, antibodies, or its nucleic acids.

The histological examination of gastric mucosa samples allows the assessment of the density of H. pylori in the gastric epithelium or the presence of gastric mucosa alterations. The H. pylori density grade is poor, moderate, or dense inflammatory infiltration in the gastric epithelium with neutrophils. Other distinctive features of chronic gastritis are the increased number of plasma cells in the lamina propria (LP) and intestinal metaplasia (IM). The HP act as virulence or protective agents. The culture of H. pylori from gastric biopsies allows the phenotypic detection of antibiotic resistance mechanisms. This method is time-consuming and less sensitive than molecular methods. Original non-invasive tests based on whole-gastric juice-based urease tests require saccharose and pH indicator 4–7.9 [10].

Culturing of gastric biopsies on commercially available selective media supplemented with antibiotics, urease, and long incubation times under specific environmental conditions allows the definitive detection of H. pylori infection. The pH varies between 6.0–8.4, making the media appropriate for H. pylori growth. However, this method requires experienced personnel, many reagents, and reading of colonies. Efforts to establish the production of black colonies thanks to certain enzymes such as urease and other virulence factors are unfruitful. The testing of drug susceptibility can be performed before or after treatment. This test confirms H. pylori infection at the time of the initial treatment of the infected patient by subjecting HP to antibiotic susceptibility testing on various media enriched in deionized water, sheeps' blood, or brain heart infusion.

6.1. Endoscopic Techniques

The relative rarity and the malignant potential of gastric lymphoma (MALToma) also fractures the paradigm of H. pylori screening and eradication [9]. For this reason their management has long been considered outside of the high risk screening initiatives. Recent studies indicate that up to 80% of the cases have H. pylori infection, in all stages of lymphomatous transformation and that successful eradication often results in histological remission. Definition of the necessary requirements for successful eradication has proved challenging and although it is likely that most fever cases can be managed non-operatively with prompt treatment, a recommendable protocol still needs to be established. Whatever the choice of method, the possibility of sampling tissue rather than just mucosal secretions opens up the new field of molecular biology techniques. Advances in techniques such as PCR enable detection of H. pylori DNA in the samples taken as well as indication of the presence of gene mutations responsible for antibiotic resistance [11]. In addition, robust technique for detection of molecular markers of pre-cancerous and cancerous conditions such as thickening of the muscle wall create the possibility of a pre-gastric cancer screening for populations with very high prevalence. Increasingly sophisticated biomolecular methods, including micro-channels for rapid polymerase chain reaction detection of diagnosis, are also currently being developed. Noninvasive molecular techniques may obviate the need for endoscopy and to provide the required tissue sampling. Overall there is not yet sufficient data to undertake either robust risk-stratification or screening initiatives with known safety and cost constraints likely to be necessary.

6.2. Non-Endoscopic Techniques

Diagnosis of H. pylori infection is conventionally performed via tissue sample examination techniques which are usually endoscopic. However, there is a growing demand for less invasive,

easy-to-administer techniques that may not require hospitalization. Non-invasive tests for H. pylori are available for more than three decades. In comparison to endoscopic methods, non-invasive tests for H. pylori infection boast an overall acceptance by the patients. Furthermore, these tests enjoy better safety since they do not deliver any undesirable side effects to the patients [11]. In addition, non-invasive tests are considerably cheaper and easier to administer. These non-endoscopic tests can be divided into 3 distinct categories such as antibody detection tests, urea breath tests, and nucleic acid detection tests.

Serological studies are among the most widely used and commercially available non-invasive tests. These tests detect specific immunoglobulin G (IgG) antibodies targeting H. pylori antigens in the serum or plasma. Serology-based tests have exhibited moderate sensitivity and high specificity in identifying currently active H. pylori infection. Unfortunately, the currently active infection may not be differentiable from a past exposure to H. pylori, or IgG antibodies may remain positive for many years after resolution of the infection. Antibody tests cannot also discriminate between naturally acquired and vaccine-induced immunity; hence assessment of past exposure to the infection is less useful.

Urea breath tests (UBT) are considered one of the preferred non-invasive methods for diagnosis of H. pylori infection. UBT measures H. pylori activity instead of the host's immune response against the infection hence great accordance with active infection. H. pylori metabolizes urea into ammonia and carbon dioxide. After oral delivery of isotopically labelled urea, breath samples are collected after a defined interval of time. The samples are analyzed for isotopically labelled carbon dioxide. Most patients require only one breath sample collection which is an additional benefit. UBT offers high sensitivity and specificity that have been demonstrated by numerous studies. The test works best 4 to 6 weeks post eradication therapy. Many potential difficulties and issues are associated with UBT. Caution must be exercised when applying UBT in patients with conditions impairing acid secretion. In addition to these, plenty of other factors are also associated with the test. [12][13][14]

6.3. Molecular Diagnostics

Molecular Diagnostic Techniques (MDTs) have emerged as an important tool for the diagnosis and prediction of antimicrobial resistance of H. pylori infection in adults and in children. The currently available non-invasive molecular approaches as well as the most promising methodologies under development are described. It also reviews the available endoscopy-based approaches that can be performed on gastric biopsy samples, which are fundamental for understanding primary resistance in all the pediatric populations [9].

Gastric biopsy is valuable for diagnosis, discrimination of active infection, and phenotypic resistance testing. However, this approach is invasive and poses limitations in a pediatric setting. MDTs based on molecular techniques have gained attention to rapidly and accurately detect H. pylori infection and its resistance to antibiotics. Consequently, performance evaluation and diagnostic accuracy metanalyses have been published in the last few years concerning stool and saliva-based molecular tests. Both methodologies have sensitivity and specificity greater than 90%. The first include the most widely used commercial tests based on PCR amplification and reverse-hybridization probes for the identification of this pathogen or its resistance to clarithromycin. The second include qPCR-based tests with real-time fluorescence detection. Other modalities, such as urinary tests or gustatory ultrasound assessment of methane production or vibrational sound, have been explored to evaluate their utility in the diagnosis of H. pylori infection [11].

Invasive approaches very accurately and rapidly identify H. pylori infection and its resistance to antibiotics. In particular, biocompatible micro- and nano-related systems coupled with spectrophotometric or electrochemical detection provide highlighted performance. The costly preparation and long time needed for biomolecular assays compared to standard histological examination explain the limited use of MDTs in clinical practice guidelines. Point-of-care testing

(POCT) resources, usually composed of a smart phone or a light-emitting diode (LED) source coupled with a box for optical detection, can be used economically instead of cost-efficacious laboratory-based approaches. POCT biosensors represent a field of great interest in diagnostics by allowing fast track test of non-invasive matrices at low cost.

7. Emerging Resistance Patterns

Antimicrobial resistance (AMR) has been recognized as one of the top 10 global public health threats facing humanity. H. pylori is categorized as a high-priority pathogen to develop new AMR strategies and discoveries. While AMR has been known for over three decades, it remains a major public health problem today, hampering successful H. pylori eradication in many regions worldwide.

Although H. pylori infections are usually asymptomatic, a higher risk of certain diseases is observed in infected individuals, and eradication of the bacterium decreases the risk of developing complications. H. pylori is the primary causative agent of 90% of gastric ulcers and 75% of duodenal ulcers. It also plays a substantial role in the development of gastric mucosa-associated lymphoid tissue lymphoma. Additionally, colonization of the organism is also associated with gastritis and gastric cancer, the third most common cancer in men and the fifth in women. Infection with H. pylori is considered a group-1 carcinogen.

It is estimated that H. pylori colonization affects roughly 50% of the worldwide population. It has been successfully eradicated in over 80% of patients who comply with the recommended regimens (a combination of at least two antibiotics with a proton pump inhibitor). These regimens were applied based on the discovery of different drug-target sites and methods of inhibiting their action. H. pylori was traditionally considered susceptible to virtually all antibiotics in the 1990s. However, treatment failure due to antimicrobial resistance (AMR) became a global problem in the following decades. [15][16][17]

7.1. Antibiotic Resistance

Recent studies on Helicobacter pylori resistance to antibiotics reveal that in the past decades, the prevalence of resistance toward metronidazole, clarithromycin, and fluoroquinolones has increased continuously. The clinical implications of these resistant strains, besides identifying their mechanism, raise public health concerns. Many countries presently consider H. pylori an important public health issue. Although an increasing prevalence of antibiotic resistance in H. pylori is widely reported, there is much heterogeneity in the reported rates among countries and geographic regions. Recently, standard clinical breakpoint values for antibiotics against H. pylori strains have been proposed to help promote harmonization in H. pylori resistance monitoring programs and facilitate further understanding of H. pylori antibiotic susceptibility testing. Such monitoring programs are expected to gain more interest and achieve greater coordination in the future.

In H. pylori, antibiotic resistance remains a significant and emerging public health issue. This, together with the adverse impact of antibiotic resistance on treatment outcomes, raises concerns about H. pylori eradication and the risk of serious sequelae, such as gastric cancer. Herein, the rise of antibiotic resistance patterns of H. pylori isolates in the past two decades and the global state of antibiotic resistance are reviewed, with particular emphasis on the clinical implications of antibiotic resistance and public health concerns regarding its rise in H. pylori. [18][3][19]

7.2. Resistance Mechanisms

Knowledge of the individual resistance mechanisms is crucial to designing successful therapeutic regimens. Natural resistance can occur due to the impermeability of the drug or active extrusion or modification. H. pylori possesses a reciprocal drug efflux mechanism that extrudes not only antibiotics but also various structure-unrelated compounds [5]. Active extrusion mechanisms also include novel drug targets for developing new inhibitors of the efflux mechanism. Traditional

antibiotics have been used to treat H. pylori infection, and new fluoroquinolone and rifaximin-induced mutations and resistance have been reported.

Acquired resistance typically occurs through mutation, horizontal gene transfer, or by chemical modification. Mutations in drug-target genes are the most common mechanism of resistance for macrolide, fluoroquinolone, metronidazole, and rifampicin. Point mutations of 16S rRNA base A to G at position 2058 or 2059 confer high-level resistance to clarithromycin by impairing binding to the methyltransferase tRNA.

Nonsynonymous mutations in the Helicobacter pylori 23S rRNA gene that confer clarithromycin resistance include A-to-G transitions and A-to-C transversions. Other resistance mutations confer 2–8-fold resistance and can act as secondary mutations leading to higher resistance. hsrM is involved in Helicobacter pylori resistance to erythromycin, and hsrM homozygous and heterozygous mutant strains were highly susceptible to erythromycin. Mutations frequently occurred in three codons, resulting in a substantial increase in the minimum inhibitory concentration of fluoroquinolones. Substitutions in the GTPase domain of the 23S rRNA gene P subunit were detected in rifampicin-resistant Helicobacter pylori.

7.3. Global Trends in Resistance

Collectively, antibiotic resistance rates of H. pylori were over 20% for all three antibiotics, with geographical differences. Most H. pylori strains exhibited resistance to amoxicillin or tetracycline. Only China reported low rates (≤10%) of fluoroquinolone resistance. Overall, the odds of treatment failure increased with the number of resistance genes detected, and an additional 0.4-0.7 months were needed to obtain a treatment success for each additional gene. H. pylori co-infected strains were more resistant to amoxicillin, clarithromycin, levofloxacin and tetracycline. Co-infection with CagA-positive and CagA-negative strains was significantly associated with higher levofloxacin resistance and treatment failure rates, respectively. All studies on primal resistance rates performed in Africa and the Americas reported resistance rates of ≤10% and ≤30%, respectively, whereas the rest of the WHO regions reported resistance rates of >40%. Among H. pylori eradication regimens, proton pump inhibitors were resistant to H. pylori infections. It was likely that the emergence of antibiotic resistance among some strains leads to treatment failure, resulting in poor prognosis. In addition, antibiotic resistance patterns varied worldwide, with the lowest clarithromycin resistance rates (>5%) reported in China and the Middle East-North Africa region. Noticeably, the resistance pattern primarily appears to depend on geography, as there are regional differences in the emergence of clarithromycin resistance with utmost minimum rates in children. Though rare, H. pylori strains could exhibit resistance to amoxicillin, tetracycline, or rifampin, but treatment failure in such cases was adequately countered by alternative regimens, and hence this phenomenon is rarely observed. Although many atypical resistance phenotypes were reported worldwide, they were rare [20].

8. Challenges in Diagnosis

The importance of investigating the emergence of previous infection is stressed due to the increasing global burden of H. pylori infection and its consequences. Limited sensitivity and robust performance have been recognized crucial weaknesses in all available point-of-care stool antigen test non-invasive methods [9]. Moreover, patient preparation before obtaining the sample remains a mandatory prerequisite. Though more recent low-cost Molecular Biology based methods, such as several types of PCR have been developed, such methods are not point-of-care tests and cannot be utilized without specific equipment and personnel skill when they are to be performed outside a laboratory environment. Therefore the presently available non-invasive diagnostic methods remain imperfect.

It has been identified that mutations associated with antibiotic resistance may be recognized in the H. pylori genome soon after infection. This insight initiates a revolution in re-thinking the design of primary therapy. Molecular biology-based methods, such as sputum-analysis might permit an

early-resistance-tailored first-line-treatment to be introduced [11]. High resistance rates against the antibiotics recommended as first-line treatment, worldwide and recently, across Europe, have been documented. This phenomenon has been greeted with pressure to de-escalate therapy to a less-effective regimen in the face of increased resistance. However, over the past two years, initial reservations about diagnosis and treatment options not based on culture have been alleviated. The fuelling of research, validation of instructions, and market introduction of various stool-based tests and tests of validity are to be hoped for future progress.

8.1. False Negatives and Positives

Helicobacter pylori colonization of the gastric mucosa leads the host to develop chronic gastritis, which may progress to more complex disease states [9]. H. pylori are genetically heterogeneous in their structure and function; precise identification of strains is not currently available, but it is expected to develop during testing of the primer sequences that amplify nucleotide regions that are hypervariable between strain types. H. pylori bloodstream invasion is possible; attempts at culturing these isolates have had limited success due to a lack of animal models that mimic H. pylori's virulence in humans. Rapid urease tests are widely used to evaluate the efficacy of antibiotic therapies against gastric H. pylori; however, false-negative test results have been observed in antrum-predominant infections. Positive tests also have false-positive test results associated with these samples in patients who H. pylori has otherwise become eradicated using antibiotics. Noninvasive molecular tests that screen PCR products using high-performance Liquid chromatography or fluorescence melting-curve analyses have been reported to detect small amounts of H. pylori in stool samples [11]. Molecular tests detect gene mutations associated with clarithromycin, amoxicillin, and metronidazole resistance in H. pylori DNA isolated from gastric biopsy or stool samples. Noninvasive stool tests that analyze A2142C and A2142G mutations have been commercialized. The aim of this review is to highlight the optimization of molecular tests that are suitable for H. pylori diagnosis and drug-resistance evaluation in the stool target. Molecular tests for H. pylori detection in mucosa or stool samples are based on conventional or real-time PCR targeting bacterial genes that amplify 188-672 bp fragments of the 16S rRNA and ureC genes. E-test broth microdilution PCR tests that identify clarithromycin A2142C, A2143G, and A2142G mutations, as well as mutant strains, have been developed. Targeted gene amplification has produced low positive and false-negative results. These results show that false-positive results are significantly associated with culture, E-test, and rapid urease test false-negative results.

8.2. Limitations of Current Tests

Accurate determination of H. pylori susceptibility to antibiotics constitutes a major clinical need guiding effective therapy for individuals with H. pylori-related disease and high-risk individuals. Culture and antibiotic susceptibility testing from gastric biopsies is the gold standard for H. pylori antimicrobial susceptibility testing. Disappointingly, culture-based susceptibility testing for H. pylori is rarely performed in clinical practice for several reasons: difficulty in obtaining gastric biopsies, increased travel for diagnostic labs, high costs of H. pylori culture and susceptibility testing, dependence on biopsy, and missed opportunities for appropriate first-line therapy. However, both culture-based and biopsy-based tests cannot address H. pylori status in individuals who were treated without obtaining gastric biopsy. To improve on these limitations, two molecular-based "non-invasive" detection strategies for resistance mutations in H. pylori have been proposed. These strategies employ stool specimens and amplify mutation(s) in genes associated with antibiotic resistance using PCR. Overall, stool-based genotypic resistance tests can replace culture-based tests for guiding therapy in subjects with known H. pylori infection if they are demonstrated to be accurate and cost-effective in clinical trials.

Molecular-based methods to detect H. pylori are rapid and less affected by specimen quality than traditional methods. The tests can be performed directly on stool, vomitus, and gastric tissues. They detect the presence of H. pylori with high sensitivity and specificity. Nucleic acid

amplification tests commonly use PCR to detect the presence of an organism and often struggle with low sensitivity because of the lack of amplification. In the presence of antimicrobial susceptibility mutations in the 23S rRNA or the gyrA, the efficacy of PCR-based tests is surprisingly high in stool specimens. The first development of stool-based PCR tests for H. pylori detection used a single conventional PCR targeting the ureC gene. Subsequently, cloning and sequencing methods were used for sequence discrimination and characterization of species. However, sequencing of PCR products has several limitations that have led to the development of newer techniques. Currently available stool-based PCR tests use various approaches for in vitro transcription of RNA or DNA from stool extracts for species classification.

9. Novel Therapeutic Strategies

In the past, the routines of first-line therapy for Helicobacter pylori eradication were almost same across the world. However, with the rising resistance to the antibiotics, the baselines have shown considerable variations based on the geography and the population [4]. Compounded by the commercialization of therapy that is scientifically not validated, there are considerable challenges in upholding the standards. With scientific rigor in treatment becoming less, the responsibility on the microbiology, and more so, on the antibiotic sensitivity, becomes even more crucial for the future of H. pylori eradication. Controlling the already rampant resistance should take the pathway of basic science, and more appropriate sequencing of therapy, with newer better drugs for the treatment of H. pylori.

There is a silver lining on the horizon. Most of the available antibiotic resistance comes from their indiscriminate uses in non-human animals. With the focus shifting to this misuse, and bacilli such as Campylobacter being the even more important dangers, H. pylori is unlikely to stay long in the radar screen of the legislatures. In line with this hope, the latest findings on candidate oligosaccharides and other non-antibiotic food substances hold promise of new avenues of H. pylori eradication. On the other hand, there is also promise from using probiotics, i.e., safe and harmless bugs that are known to kill H. pylori, bolstering the effector immune responses against it. While lactobacillus has come to mainstream with the marketed versions, enormous strains and bugs can still be sought for further refinement.

With time, it is least likely for the whole virulence to be lost by a successful bacillus. Acquisition of resistance is intimately linked to treatment pressure, i.e., if H. pylori is allowed to survive without being exposed to antibiotics, the selection of resistant strains is unlikely. Hence, treatment regimens should be designed keeping this in mind to include no more than 2 of the classes of antibiotics that are being used. New regimes should be designed for stopping with fluoroquinolones after including it in two consecutive regimens, after the introduction of Quadraplex. Prevention should still be number one priority, and any new culture facility should include the basic strains of that region and in-depth characterisation including phylogeny, virulence, antibiotic sensitivity, and resistance mechanisms. [21][22]

9.1. New Antibiotic Regimens

Helicobacter pylori is a Gram-negative bacillus. It has a helical shape and a flagellated structure that can colonize the human stomach. Both chronic gastritis and peptic ulcer disease have been linked to H. pylori infection. H. pylori is designated as a global priority pathogen because it is one of the most prevalent infections in humans. A significant and prevalent infectious disease worldwide caused by H. pylori continues to afflict at least half of the world's population. There have been rising worries regarding prevalence and drug resistance since the beginning of the twenty-first century. Nonsteroidal anti-inflammatory medications (NSAIDs), smoking, alcohol, and stress can all be risk factors for H. pylori infection and the subsequent progression of peptic ulcer disease in addition to H. pylori infection [4]. Infection control plays a crucial role in community preventive work, with ongoing concerns regarding medication treatment and antibiotic choice.

The treatment of H. pylori infection is becoming increasingly challenging due to the rise of antibiotic resistance. Antibiotic-resistant H. pylori is a global priority pathogen because antibiotic resistance is increasing globally for both gastric cancer and peptic ulcer disease. In recent years, this bacterium has become resistant to all antibiotics, including amoxicillin, which was assumed to be a core effective antibiotic for H. pylori treatment. Amid the antibiotic-resistance outbreak, non-antibiotic therapies should be included in treatment strategies and ongoing studies should find ways to restore the efficacy of antibiotics.

The treatment of H. pylori infection rises from acid suppression therapy. Over the past few decades, the treatment has transitioned from an era of single therapy acid suppression to bismuth salt therapy, sequential therapy, lactobacillus probiotics, from the standard triple therapy to the current quadruple therapy acid suppression and a combination of antibiotics. Recently, with the emergence of antibiotic resistance, it is becoming increasingly complex to treat this infection. Clarithromycin was once called the "gold standard" to assess antibiotic resistance, but vancomycin is emerging as the "gold standard." Historically, understanding the mechanism of drug-resistant H. pylori has been the focus of assent statement studies and efforts.

The results to reduce resistance include clarithromycin, metronidazole, amoxicillin, and levofloxacin. In Asia, clarithromycin resistance can change the efficacy of treatment regimens containing amoxicillin and levofloxacin; individualized treatment regimens are recommended for further studies on H. pylori resistance. Combined with the evidence from nationwide studies on H. pylori infections, resistance patterns have collapsed efficiently and safety in repaired therapy regimens.

9.2. Adjunctive Therapies

While direct antibiotic resistance has been and still is a significant barrier in eradication, antibiotic reserve may help manage treatment-resistant infections. AMPs are expect to reconstruct the metabolic pathway and expression and analyze the effects of antimicrobial peptides on resistant H. Pylori [4].

CYPs augmented with volatile solid waste as substrate is suggest at lab-scale to minimize costs with the potential on an industrial scale.

The important components of a vaccine must be elucidate. These include developing adjuvants, incorporating mucosal routes of administration, and understanding the immune evasion, suppression, and antagonism dynamics in H. pylori infection. However, most conventional vaccines fail to protect in a widespread manner due to strain diversity, antibiotic resistance, and infection-associated mutations.

The ideal vaccine should induce surface-bound protective antibodies. Ideal delivery platforms include plant-based viral-like nanoparticles, engineered Lactococcus lactis, or naturally occurring Epigallocatechin-3-gallate/silk fibroins as bioengineered exosomes. While these provide great hope in H. pylori control, few are in usability. Solutions in the pipeline may include delivery vectors or adjuvant systems with a gut-targeting property, preferably aiming at CD103+ dendritic cells or M cells, leading to more robust, long-lasting, and broadly reactive intestinal and systemic immunity.

9.3. Vaccination Approaches

Given the observed trends toward a decline in the effectiveness of H. pylori eradication regimens, there has been an intensive search for a safe and effective vaccine against H. pylori for both the treatment and prevention of infection. The development of a vaccine would be a breakthrough discovery in the field of H. pylori, with the potential for huge public health and economic benefits. The ideal vaccine would be safe and immunogenic, providing lifelong immunity, be easily produced and administered, and induce protective mucosal immunity against pathogen entry subsequent to vaccine dosing [4]. Several different types of vaccines have been studied against H.

pylori, including live attenuated, subunit, and DNA vaccines. To date, however, there is no licensed vaccine against infection.

A number of humoral and cellular immune responses to H. pylori have been reported in animal models of infection and in infected humans. However, humoral immune responses are the primary focus for most vaccines due to immunity elicited against most pathogenic microorganisms when vaccinated or infected. Antibodies have thus been reported against a plethora of different antigens including the BabA adhesin/flavoprotein, the VacA cytotoxin, the protein CagA, the SOD, the ureases, and various other components of the H. pylori cell envelope including the Hsp60 and LPS [8].

Many of the best studied H. pylori antigens involved in eliciting humoral immunity against infection and their use in vaccine construction have been recently summarized. The majority of studies to date have utilized mail urases in preclinical vaccine models. Recombinant urease vaccine constructs have induced enhanced acidity tolerance and decreased load of colonized H. pylori with up to 90% protection against challenge as well as reductions in gastric inflammation and pathology. However, gastric acidity neutralizing activity is only slow to develop and long-term administration can lead to adverse effects, thus limiting application for chronic states in infected adults. With this in mind, the protective efficacy of immunization with plant-derived recombinant ureases has been tested in several models of H. pylori infection.

10. Conclusion

H. pylori is a spiral-shaped gram-negative bacterium that can colonize the human stomach and is associated with chronic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer. Among the major human pathogens, H. pylori infection is the most common chronic infection. The World Health Organization (WHO) has classified this pathogen as a global priority pathogen, as its prevalence is still increasing, especially in developing and underdeveloped countries, and due to the increasing prevalence of antibiotic resistance. The increasing prevalence of antibiotic resistance to H. pylori has made the treatment of this infection complicated and raised the need for new and effective treatment strategies. The bacterium can typically be completely eradicated with a combination of two or three antibiotics and one acid suppressant, leading to rapid improvement of symptoms and prevention of complications, but through time, antibiotic resistance has become a significant problem that could drastically reduce the efficacy of antibiotic treatment. H. pylori infection typically takes place throughout a combination of antibiotics, and pyruvate is one of the most commonly used antibiotics to treat H. pylori infections. Since it was found that 85% of patients with H. pylori infection had pyruvate-resistant genes, impairment of H. pylori eradication was observed by the high prevalence of pyruvate resistance increasing from 56.25% to 92%. Gastric Cancer is the most deadly malignancy worldwide, and H. pylori infection is probably the most important preventable cause of gastric cancer. Recently, the American College of Gastroenterology Guidelines recommend testing for and treating H. pylori infection as an important part of the strategy to reduce the burden of gastric cancer. Treatment of H. pylori infection consists of two groups of drugs: acid suppressants and antibiotics. The objective of this review is to summarize the challenges and recent advances in the therapies of H. pylori infection.

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