Helicobacter pylori and the Art of Antibiotic Resistance; A Microbial Twilight

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Abstract— the modern society is facing an alarming rise in resistance against current antibiotics which has rattled the chances of survival against various pathogens. The emergence of diversity in resistance against drugs employed to cure gastric issues solely directs to single villain; Helicobacter pylori. This gastric manic silently resides in stomach of more than 50% global population and has devise strategies to cater impact of curative antibiotics. Studies from many countries are pointing towards increasing resistance rates against common regimens such as Metronidazole, Clarithromycin and Amoxicillin. The ability to acquire mutations has led to the modification of various antibiotic targets in Helicobacter pylori. The increasing number of treatment failure reports signifies devastating potential of Helicobacter pylori. It is need of hour to look towards smart diagnostic and pre-treatment resistance profile based antibiotic therapies. Moreover, search of new regimens against Helicobacter pylori is a vast domain which can allow human race to tackle with this rising global threat.

Key words— Helicobacter pylori, Antibiotic resistance, Peptic ulcer, Pathogens, Metronidazole, Clarithromycin, Amoxicillin

1 Introduction

Among global pathogens, the highly prevalent one is *Helicobacter pylori* which is known to colonize around 50% of the global population (1). Scientific world can never forget tremendous contributions of Warren and Marshall who first identified this peptic maniac in gastric biopsies in 1983. The *Helicobacter pylori* are characterized as Gram-negative bacilli. This pathogen has ability to infect human stomach mucosal area. The main disorders associated with *Helicobacter pylori* include diseases of the upper GIT (gastrointestinal tract) including gastric marginal zone/mucosa-associated lymphoid tissue (MALT) lymphoma, peptic ulcer disease, chronic gastritis and gastric cancer (2, 3). Recent studies have revealed that *Helicobacter pylori* might have contributions in development of extra-intestinal diseases. Disorders including refractory iron deficiency anemia, immune thrombocytopenic purpura, and vitamin B12 deficiency have found connections with *Helicobacter pylori* as shown in Figure-1 (1, 4, 5).

Epidemiology of *Helicobacter pylori*:

The epidemiological picture of *Helicobacter pylori* infection is vivid. Majority of studies have pointed towards consumption of unhygienic/ contaminated food as source of *Helicobacter pylori* infection. The probable person-to-person route of transmission of *Helicobacter pylori* includes oral-oral, faecal-oral or gastro-oral exposure. This puts major emphasis on improvements in sanitation, living environment to reduce spread of infection (6, 7). Another source of *Helicobacter pylori* are mammals and insects including cats, sheep, pigtailed monkeys and cockroaches. Apart from above mentioned reasons, another scenario is maternal infection. Moreover, socio-economic status is also a major contributor towards pediatric infection (6, 8, 9).

Prevalence of Infection:

Back in 1990s, more than 80% eradication rate for *Helicobacter pylori* was achieved. Currently, the eradication rate is dropping globally (9). In many countries, the eradication rate is decreased to 60%, owing to immense increase in antibiotic resistance all around the globe. In recent Maastricht recommendations, it is clearly directed that susceptibility testing be conducted before administering regimens in areas which have higher rates of clarithromycin resistance. Moreover, the KGCM (Kyoto Global Consensus Meeting) has declared that therapeutic compounds showing eradication rate of 90% in specific area should be employed as frontline therapy. It is prime objective now-a-days to design therapeutic compound that result in 100% cure rate (9).

The association of impact of decline in socio-economic status with risk of *Helicobacter pylori* infection has been discussed by many studies. Moreover, age related cohort studies have claimed that prevalence of disease increases with age. Among European countries, the prevalence rates of

Helicobacter pylori infection range from 11% to 60.3% with lowest in Sweden and highest in Spain respectively (9-11). On other hand, *Helicobacter pylori* prevalence has been reported to be 83.4% in China (12, 13). Since last 20 years, the *Helicobacter pylori* has increased in countries like China, Japan and Bulgaria (14, 15). Varying rates of *Helicobacter pylori* infection in Canada have been reported with 30% in local population but almost 95% in Aboriginal populations living in Canada. An overall 30% seropositivity rate has been observed in studies conducted by National Health and Nutrition Examination Survey (NHANES) III and NHANES 1999–2000 in USA. (1) It has been suggested that multiple strains of *Helicobacter pylori* are the reason of high infection rates (16-18)

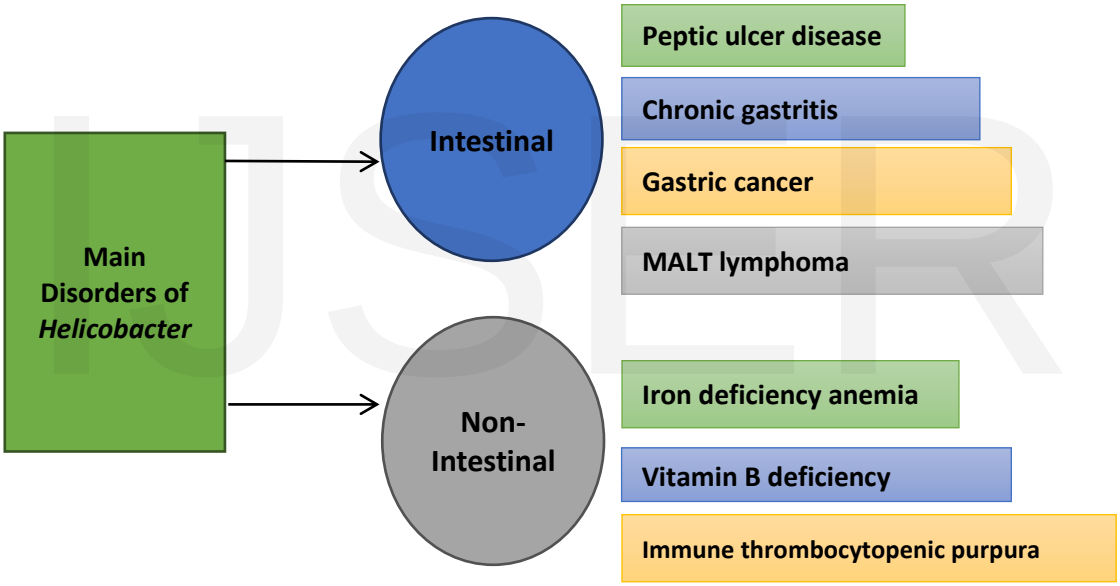

FIGURE-1: Disorders associated with *Helicobacter pylori* infection

Prevalence of Antibiotic Resistance:

There are many reasons that explain the decline in anti-microbial based eradication rates of *Helicobacter pylori* since last few decades; among them the major cause was found to be development of resistance in *Helicobacter pylori* and non-compliance of patients (19, 20). In a European study that included 2204 patients across 18 countries from 2008-2009, it was clearly established that the resistance in *Helicobacter pylori* against Metronidazole, Clarithromycin and Levofloxacin have raised to 34.9%, 17.5% and 14.1% respectively (21). In Japan, the National Surveillance study took 3707 *Helicobacter pylori* samples during 2002-2005. It was established by Kobayashi et al. that the rates of clarithromycin resistance in *Helicobacter pylori* increased from 18.9% to 27.7% during 3-year duration. Moreover, Metronidazole resistance rate appeared constant, with ranging from 3.3% to 5.3%. On other hand, Amoxicillin appeared to be most potent inhibitor of *Helicobacter pylori* with negligible resistance rates (22, 23). In another study, the antibiotics which are integral part of therapy against *Helicobacter pylori* were checked for resistance.

Surprisingly, resistance against Metronidazole (36.9%) was highest as compared with Clarithromycin (10.1%) and Amoxicillin (1.4%) (24). An extensive study was conducted by HARP (Prospective Multicenter *Helicobacter pylori* Antimicrobial Resistance Monitoring Program) encompassing resistance rates of *Helicobacter pylori* during 1998 and 2002. The finding of this study was similar to previous ones with highest resistance against Metronidazole (25.1%), followed by Clarithromycin (12.9%) and least resistance

against Amoxicillin (0.9%). A major factor in development of antibiotic resistance is believed to be overall consumption of antibiotics in a population (24). A study was conducted on Alaskan Native population in which it was established that patients with prior consumptions of Macrolide were having clarithromycin resistant *Helicobacter pylori* infection. About 92% of patients which had consumed Macrolide previously were infected with clarithromycin resistant *Helicobacter pylori* (25).

Clarithromycin Resistance:

Antibiotic resistance studies have been performed to assess impact of *Helicobacter pylori* resistance against various antibiotics. The major concern of these studies was Clarithromycin resistance as it has been reported in many countries. Studies have established that enhanced rates of *Helicobacter pylori* resistance against Clarithromycin are linked with high *Helicobacter pylori* sero-positivity. In Japanese population, clarithromycin resistance was reported to be 1.8% in 1996 which increased to 27.1% in 2008 (26). Okamura et al. reported that during 2000-2013, there was resistance rate of 31.1% (27). Consequently, an approximate increase from 40% to 50% in *Helicobacter pylori* sero-positivity was observed (14, 27). In China, during 2000-2014, the increase in Clarithromycin resistance has been reported as 14.8% to 52.6%. Similarly, the sero-positivity rates of *Helicobacter pylori* increased from 65% to 85% (28-30). In Korea, from 2005 to 2009, the rate of clarithromycin resistance increased from 11% to 60% respectively. A study based on population of USA revealed that during 1993 to 2002, the rate of clarithromycin resistance increased from 6.1% to 12.9% (31).

Metronidazole Resistance:

A wide range (20% to 60%) of Metronidazole resistance in *Helicobacter pylori* have been reported for European and USA population. The only exception in this regard is Northern Italy with only 14.9% resistance against Metronidazole (32). In Europe, 33.1% resiatnce rate against metronidazole has been reported. Moreover, the central and eastern regions of Europe had comparatively lower resistance than rest of European regions (33-35).

In japan, the resistance against metronidazole is 9%-12%, lowest among all other countries (36). In Canada, the reports have indicated resistance rates to be 18% to 22% (37). In recent studies, the US population had metronidazole resistance rate of 21.5% (38). A possible explanation to varying rates of resistance in various countries is difference in prior use of metronidazole. It is evident from studies on Alaskan population that people with prior use of metronidazole had higher rate of *Helicobacter pylori* resistance towards metronidazole (25, 39).

Other Antibiotics:

A study has concluded that rate of Tetracycline resistance in Spain is only 0.7% while 0.5% both in UK and Hong Kong (40, 41). There are limited studies related to rate of resistance against fluoroquinolones. Resistance against Levofloxacin in *Helicobacter pylori* has increased markedly as evident from studies in China and Italy where the prevalence of Levofloxacin resistance was reported to be 34.5% and 22.1% respectively (30, 42). The increase in resistance against Levofloxacin has affected USA as well with 31.9% resistance rate (38). A study conducted on 110 adult patients in Portugal reported 20.9% resistance against Levofloxacin (43). A resistance rate of 4.7% was reported against Trovafloxacin in Netherlands. This drug has yet to introduce into local Dutch market. This result clearly confers development of cross resistance among variety of antibiotic molecules of same group (44). Moreover, in Eastern Europe, five countries reported to have 3.9% rate of resistance except France where 3.3% resistance was reported (45, 46).

It has been well established that Sitafloxacin resistance has no link with prior use of fluoroquinolones. Surprisingly, this drug has achieved higher *Helicobacter pylori* eradication rates. In case of Amoxicillin, almost negligible resistance (0-2%) has been reported in Dutch community and Germany (47). About 6% rate of resistance in *Helicobacter pylori* has been reported in Alaskan population against Amoxicillin (25). However, Amoxicillin resistance rates in Asia and South America were reported as 38%, although reports suggest that *Helicobacter pylori* resistance

against Amoxicillin has no impact on treatment (48, 49).

Diagnosis of *Helicobacter pylori*:

Since last decade, for patients of dyspepsia and epi-gastric pain, *Helicobacter pylori* testing has become a routine tool (50). As per guidelines of International Agency for Research on Cancer, *Helicobacter pylori* is declared as Group-1 carcinogen. The eradication of *Helicobacter pylori* ultimately decreases gastric carcinoma and MALT lymphoma cases (51, 52). Currently, there are two methods to look for *Helicobacter pylori*; they are nomenclature as invasive and non-invasive methods. The major aspects that differentiate these methods are age of patient and kind of symptoms apart from types of each method (53). For patients of age group below 55 years and dyspepsia, detection of *Helicobacter pylori* is done via non-invasive method (54, 55). Among non-invasive methods, peripheral blood serology, stool antigen test and urea breath test are included. The immunoglobulin G antibodies to *Helicobacter pylori* are detected by serological testing. However, in current guidelines, serological tests are not recommended as sero-conversion for *Helicobacter pylori* is not observed often (56, 57). Moreover, the serological tests don't work for post-eradication monitoring as IgG levels are not altered immediately after treatment (58). Active infection can be detected by serological techniques in case of IgM antibodies against *Helicobacter pylori*. The only issue is the levels of IgM raise for short duration after infection (59). Now-a-days, the gold standard for detecting *Helicobacter pylori* is Urea breath test. The mechanism involves detection of urease activity of *Helicobacter pylori* via 13-C/14-C radioactive carbon. This test is commonly used for post-treatment monitoring (60). Monoclonal and polyclonal anti-*Helicobacter pylori* antibodies are used to detect *Helicobacter pylori* antigen in stool via Stool antigen test (61). This method has potential of post-treatment monitoring (62). Stool antigen test is recommended in areas where UBT (urea breath test) is unavailable (53, 63). In case of alarming symptoms such as sudden weight loss, progressive dysphagia, gastrointestinal bleeding, sudden iron deficiency, an invasive procedure, upper endoscopy is recommended (54). In case of Patients with age 55 years or above age, upper endoscopy is recommended (54).

Rapid urease is recommended if biopsy samples are easily available. For this purpose, biopsy samples from gastric antrum and body are collected for detection of urease (64). Many staining methods are available for histological evaluation of tissue sections. These methods include specialized stains such as modified Giemsa or Warthin– Starry and *Helicobacter pylori* immune-histo-chemical stains (65).

Antibiotic-Based Therapeutic Approaches

In order to treat *Helicobacter pylori* infection, various therapeutic options have been developed as shown in Figure-2. Salvage therapy is recommended in case of failure of other treatments (66).

First-Line Therapy: Many biological and non-biological factors such as cost, antibiotic strength, duration, side effects and tolerability of drugs, bacterial resistance and local antibiotic use determine impact of antibiotics. In certain areas where <15% clarithromycin resistance is reported, PPI-based triple therapy, which includes PPI, amoxicillin and clarithromycin, is recommended regimen for *Helicobacter pylori* treatment (67). Metronidazole is a recommended substitute for amoxicillin where penicillin allergy. Studies have revealed that major cause of failure of eradication of *Helicobacter pylori* infection by PPI-triple therapy is antibiotic resistance to Clarithromycin (68).

Apart from factors mentioned above, many other attributes such as patient compliance to antibiotic, body weight of patient, type of *Helicobacter pylori* strain, bacterial load and gastric acidity do affect cure rates. In case if *Helicobacter pylori* resistance to antibiotics increases from 20%, then sensitivity testing is recommended by Maastricht guidelines (69).

As per resistance against clarithromycin, 7-day administration of triple therapy is recommended in case of 15% resistance rates while 14-days treatment for more than 20% resistance against clarithromycin (67, 68).

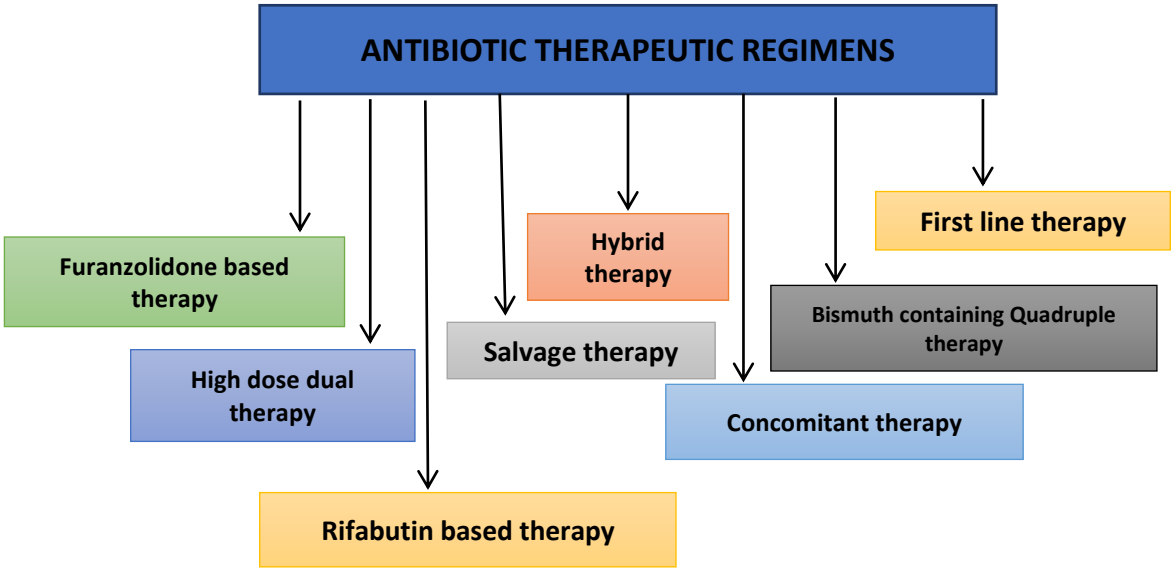

FIGURE-2: Available regimens against *Helicobacter pylori*

Bismuth-Based Quadruple Therapy: The composition of Bismuth-based quadruple therapy includes bismuth, PPI and at least two antibiotics, mainly metronidazole, tetracycline and clarithromycin (70). For areas having high levels of resistance against clarithromycin and metronidazole, this regimen is considered as first line treatment approach. The ability of this regimen to overcome resistance against both clarithromycin and metronidazole makes it first choice therapeutic approach (70). There are many countries which lack bismuth salts and tetracycline. As a substitute, Doxycycline or amoxicillin could be used (71). However, results have indicated limited compliance due to frequent drug dosage (72).

Sequential Therapy: Zullo et al. introduced Sequential therapy which is constituted in 2-phases. The first phase includes 5-day administration of PPI & amoxicillin while 2nd phase includes 5-days administration of PPI, metronidazole and clarithromycin (53). In case of high rates of clarithromycin resistance or due to penicillin allergy, Levofloxacin could be employed as an alternative. Studies have revealed that amoxicillin deactivates clarithromycin resistance mechanisms in *Helicobacter pylori* by disrupting The only problem is patient compliance as complexity of this therapeutic approach has reportedly shown decrease in suitability of this regimen with patients (74). *Helicobacter pylori* cell wall and ultimately causing deactivation of efflux channels (73).

Moreover, in case of treatment failure, risk of emergence of multi-drug resistance is on cards. Likely, the consumption of main antibiotics, which are effective towards *Helicobacter pylori*, in sequential therapy narrows window for salvage therapy (74).

Concomitant Therapy: The Concomitant therapy involves administration of PPI, amoxicillin, clarithromycin and metronidazole for duration of 10 days. Studies have shown superiority of this regimen over triple therapy. More than 90% PP (per protocol analysis) and more than 80% ITT (intention-to-treat) eradication % in case of concomitant therapy have been revealed in a meta-analysis which encompassed nine studies that were conducted in Germany, UK, Spain, Japan and

Italy (74). In Taiwan, an evaluation study based on sequential therapy and concomitant therapy including levofloxacin, metronidazole, PPI and amoxicillin reported resistance rates of 0.6% (Amoxicillin), 10.2% (Levofloxacin), 6.6% (Clarithromycin) and 33.5% (Metronidazole). The levofloxacin used in concomitant and sequential therapy showed 92.2% and 93.3% eradication rates respectively in ITT analysis (75). The ability to overcome dual antibiotic resistant microbes and compatibility with patients makes concomitant regimen preferable over sequential therapy (76). The effectiveness of concomitant regimen relies on prevalence of antimicrobial resistance in *Helicobacter pylori* which is a variable factor geographically (76).

Hybrid Therapy: A hybrid therapy based on combination of concomitant and sequential regimens was proposed by Hsu et al (77). The Hybrid therapy consists of two phases: 7-days administration of amoxicillin and PPI followed by concomitant quadruple regimen including amoxicillin, metronidazole, clarithromycin and PPI for 7 days. In ITT analysis, 97% eradication rate was observed with this treatment while 99% eradication rate was observed in PP analysis. A recent study has indicated effectiveness of hybrid therapy is equivalent to concomitant therapy of 14-days (77).

Salvage Therapy: In salvage regimen, use of levofloxacin is recommended. In second line therapy, triple therapy containing levofloxacin is most recommended regimen (78). The salvage therapy is administered for 10 days constituting of amoxicillin, levofloxacin and PPI. Variable eradication rates have been reported in Europe and Taiwan (65-96%). In another study conducted by Basu et al., 653 patients were administered with LOAD (Levofloxacin, Omeprazole, Nitazoxanide and Doxycycline) for 7-10 days. It was concluded that LOAD (90%) had higher efficacy as compared with standard therapies (73%). For patients who have failed *Helicobacter pylori* eradication with first-line and second line therapies, Levofloxacin-based regimen is considered as empiric third-line therapy (78). A study emphasized on use of combination of levofloxacin, rabeprazole, bismuth citrate and amoxicillin for 10-days. This treatment achieved 84% eradication rate against multi-drug resistant *Helicobacter pylori*. However, patients with resistance against levofloxacin and amoxicillin showed decreased eradication rates (79).

It must be ensured that quinolone therapy is selected on the basis of antibiotic susceptibility testing and geographical patterns of resistance. The main reason behind this is the emergence of quinolone resistance due to increased utilization of quinolones in treatment of respiratory infections and UTIs. Moreover, due to frequent use of quinolones in other therapeutic regimens, this therapy is not employed as first line therapy. Now-a-days, in areas with <10% quinolone resistance and >20% clarithromycin resistance, this regimen is employed as second-line therapy (80).

Rifabutin Based Therapy: In-vitro studies have revealed promising results of *Helicobacter pylori* eradication by rifabutin based therapeutic regimen which further contains rifabutin, PPI and amoxicillin. The problem, however, is lack of defined duration of treatment which is currently 7-days, 10-days and 14-days as well. Moreover, another important complication is rare myelotoxicity which should be addressed before public administration of rifabutin (81). Due to rapid rise in antibiotic resistance, cross resistance in mycobacteria can emerge which limits use of this therapy only for rescue purposes. For areas where bismuth and tetracycline are not available, this regimen can be employed as third-line therapeutic approach (82).

Furanzolidone Based Therapy: In case of failure of second-line therapy, Furazolidone-based regimen is employed for a week which contains tetracycline, furazolidone lansoprazole and tri-potassium di-citratobismuthate. Due to emergence of cross resistance with metronidazole and increasing reports of side effects, this regimen is not administrated commonly (83, 84).

High Dose Dual Therapy: For areas of high rates of clarithromycin resistance, a High-dose dual therapy has been introduced. 14-days administration of PPI and amoxicillin, thrice a day, makes it potent inhibitor of *Helicobacter pylori* (85). A study was conducted to compare this regimen with standard triple therapy. The ripple therapy, composed of 1000 mg amoxicillin, 500 mg

clarithromycin & 30 mg lansoprazole b.d. for 14-days resulted in 82.8% eradication rate while this regimen administrated for 14-days containing 750 mg amoxicillin with 30 mg lansoprazole resulted in 78.4% eradication rate of *Helicobacter pylori* (86).

Antibiotic Resistance Mechanims

Amoxicillin: An important member of penicillin family is Amoxicillin which is beta-lactam, moderate-spectrum bactericidal antibiotic. The *Helicobacter pylori* develop resistance against amoxicillin by altering penicillin binding proteins and decreasing antibiotic access into bacterial cell. An important mechanism against beta-lactams is point mutation in pbp1A gene. Moreover, development of membrane based efflux pumps is also resistance mechanism. In *Helicobacter pylori* strains, resistant to amoxicillin, have mutations in hefC, hofH, hopC and pbp2 (47).

Clarithromycin: Clarithromycin reversibly binds with 50S ribosomal units to inhibit protein synthesis in bacteria. The components of 50S ribosomal unit include 5S ribosomal RNA, 23S ribosomal RNA and RNA binding proteins. The target site of clarithromycin is the peptidyl transferase which is located at V domain of 23S ribosomal RNA. Point mutation in 23S rRNA gene is major contributor of resistance against clarithromycin. The most frequent mutation in 23S rRNA gene is A2143G (69.8%). Moreover, 11.7% mutations have been reported as A2142G while only 2.6% mutation reported is A2142C (33). The major impact of these mutations is prevention of binding of antibiotic with 23S ribosomal RNA. Apart from these, other contributors towards clarithromycin resistance include mutations such as C2147G, T2190C, A2115G, A2223G, G2141A, C2195T and C2694A (87).

Metronidazole: Metronidazole, a synthetic nitroimidazole, is a bactericidal antibiotic. The mode of action of this antibiotic encompasses production of a toxic metabolite via action of nitro-reductases in cytosol of bacteria. A major contributor towards resistance against metronidazole is mutations in rdxA. The development of mutation in oxygen-insensitive NADPH nitro-reductase leads to development of resistance, as this enzyme is required for activation of this drug. Apart from these, other genes such as fdxB (ferredoxin-like enzyme) and frxA (NADPH flavin-oxido-reductase) have also contributed towards metronidazole resistance (47).

Tetracycline: Tetracycline acts by inhibiting protein synthesis in which this bactericidal antibiotic binds with 30S subunit of ribosomes. This binding blocks further attachment of aminoacyl-tRNA which ultimately leads to halt in nascent peptide formation. The resistance mechanism against tetracycline involves action of efflux pumps which actively efflux antibiotic from bacteria. It has been reported that deletion of efflux channels enhances tetracycline sensitivity (86).

Moreover, ribosomal protection proteins also confer resistance against tetracycline. The mode of action of these proteins involves removal of attached antibiotic from ribosome. Additionally, these proteins alter affinity of ribosome for attachment of tetracycline. Apart from these, reports suggest that point mutations in 16S rRNA gene and tetracycline inactivation by enzymes are also contributors towards resistance against tetracycline (86).

Levofloxacin: In case of resistance against quinolones, the quinolone-resistant determination region plays an important role. It has been reported that point mutations in genes that encode DNA gyrase (gyrA) have been identified at position in the codons that code for amino acids including 87, 88, 91 and 97. These mutations confer resistance against levofloxacin and other quinolones. Recent studies indicated the ability of sitafloxacin to overcome the resistance established by these mutations (88, 89).

Detection of *Helicobacter Pylori* Resistance

Culture Based Methods

For areas of high clarithromycin resistance, endoscopy based susceptibility testing techniques are employed for both before application of first-line therapy as well as after second-line regime has failed to eradicate *Helicobacter pylori*. Now-a-days, there are many methodologies employed to determine *Helicobacter pylori* antibiotic resistance but the sole difference is time frame and nature of sample (90, 91).

For large number of strains of *Helicobacter pylori* most common method is agar-dilution technique. But this technique is not recommended for small

number of samples (92). Another method, known as E-test, involves application of test strips on agar plate, inoculated with *Helicobacter pylori*, which evaluates MIC (Minimum Inhibitory Concentration) of test drug (87). It has been reported that reproducible results can be obtained from E-Test especially in case of *Helicobacter pylori's* sensitivity towards metronidazole, ampicillin and clarithromycin (93). It has been internationally established that E-Test is most suitable methodology for determining *Helicobacter pylori* sensitivity. Only issue is the lack of global availability of clarithromycin's E-test strips (94). Among drawbacks of these methods is ability to screen one strain at a time. This ultimately leads to failure in provision of resistance profile of *Helicobacter pylori* in areas where multi-strain resistance is common (93).

Molecular Techniques

The phenotypic methods such as agar dilution methods, performed to assess antibiotic resistance, are gold standard methods. However, a limiting factor in these methods is time factor as these methods require 7-14 days for completion of procedure. In order to decrease turn-around time, molecular methods for detection of mutations were developed. Moreover, molecular methods can employ both fresh and malin-fixed samples for processing (95, 96).

In order to determine *Helicobacter pylori* susceptibility towards clarithromycin, Real-time PCR has been used successfully (97, 98). Moreover, 23S rRNA mutations in *Helicobacter pylori* has been detected by PCR from formalin-fixed paraffin-embedded samples. From patients infected with multiple strains of *Helicobacter* pylori, PCR can retrieve maximum antimicrobial resistance data. PCR oriented methods are clearly spoiled by DNA contamination/ degradation which questions the ability of these methods to rapidly detect microbes. Moreover, reports have suggested that at many occasions PCR based methods resulted in detection of non-cultureable microbes or dead microbes (99).

Another important, time saving, economical and accurate molecular method is FISH (Fluorescence in situ hybridization). This method is employed to detect antibiotic resistance pattern of *Helicobacter pylori* colonies. The ability of this technique to directly detect *Helicobacter pylori* resistance from biopsy samples, which are prepared for microbiological and histo-pathological examination makes it effective tool. Most importantly, this technique can provide results within 03 hours of endoscopy (100).

Another important aspect of FISH is DNA molecular probes which are employed for bacterial detection (101). In this method, PNA molecules are used which are mimics of DNA and have high affinity for DNA and RNA (102). Due to small size, almost 13-18 nucleotides, PNA molecules can easily enter through cell wall of bacteria. PNA molecules have more ability to resist nucleases and proteases as compared to normal DNA molecules (103).

2 Discussion

Peptic ulcer is growing worldwide and has established geographical variations. The main culprit, *Helicobacter pylori*, has developed strong antibiotic resistance profile which is increasing with time. In Asian countries like China, Japan and Korea, since past 20 years, the antibiotic resistance has paced and currently above the threshold limit of 15-20%. Meanwhile, during same time frame, the eradication rates of *Helicobacter pylori* have dropped by empiric regimens (104).

The failure of treatment involves many factors which still require lot of research. Among them most contributing are antibiotic resistance and poor compliance. Most importantly, very less data is available in terms of re-infection which influences treatment failure measurement. Moreover, variations in antimicrobial susceptibility of various *Helicobacter pylori* strains found in stomach also enhance treatment failure (47, 105).

Currently, scientific world must pay attention towards this alarming situation where antibiotic resistance rates are reaching to critical limits and treatment failure is increasing day by day. It must be noted that the resistance rates in local populations of many countries are not available, which need to be determined. This will not only aid in proper prescription for treatment but also help in individual based antibiotic susceptibility analysis before commencement of treatment. Finally, antibiotic regimens based on patient's

requirements will not only reduce the rate of treatment failures but also halt the increasing antibiotic resistance rates of *Helicobacter pylori* around the globe.

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