Drug Resistance in *Helicobacter pylori*

Shamshul Ansari and Yoshio Yamaoka*

Department of Environmental and Preventive Medicine, Oita University Faculty of Medicine, Japan

Submission: October 24, 2017; Published: November 14, 2017

*Corresponding author: Yamaoka Y, Department of Environmental and Preventive Medicine, Oita, University Faculty of Medicine, Japan, Tel: +81-97-586-5740; Fax: +81-97-586-5749; Email: yyamaoka@oita-u.ac.jp*

Abstract

*Helicobacter pylori* being worldwide in distribution can cause several gastric complications such as gastritis, peptic ulcer diseases and gastric cancer. The risk for the development of gastric complications may be decreased by eradicating the bacteria by antibiotic treatment. However, high rate of treatment failure has been observed, primarily because of the point mutations observed in the antibiotic targets. In this review, we have briefly summarized the resistance mediated by the point mutations of common antibiotic targets.

Keywords: Antibiotic resistance; *Helicobacter pylori*; Mutations

Introduction

*Helicobacter pylori* (*H. pylori*) is a stomach pathogen that colonizes approximately 50% world’s population [1]. However, the prevalence of *H. pylori* infection tends to differ both within and between the countries. The higher infection rate up to 80% in developing countries in comparison to 20-50% infection rate in developed countries reflects the dependence of prevalence of infection on socio-economic status of the countries [2,3]. The exact route of transmission of this bacterium is not known yet but evidence supports the fecal or oral transmission of bacterium from person to person between the family members [4-6]. Once the bacterium enters the stomach it causes chronic infection because it is highly adapted to the environmental stresses [7]. Once the chronic infection is established it can lead to the several gastrointestinal diseases such as gastritis, peptic ulcer, gastric cancer and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [8]. *H. pylori* infection is the primary risk factor for the development of gastric cancer which is the third highest cause of cancer morbidity worldwide [9]. The risk of peptic ulcer and gastric cancer development may be reduced by successful eradication of *H. pylori* [10,11]. However, the increasing antibiotic resistance in *H. pylori* has posed an emerging challenge to achieve the goal of eradication therapy [12].

Therapeutic Regimens

Although many therapeutic regimens such as amoxicillin, clarithromycin, levofloxacin, tetracyclin, metronidazole are in current use either alone or in combinations but the effective antibiotic therapy seems to be difficult worldwide due to the resistant mediated failure which is the main factor affecting the efficacy of current therapeutic regimens [13]. The consumption of antibiotics in general populations reflects the prevalence of antibiotic resistant bacteria which differs in different geographic areas [14,15]. Currently, triple therapy based treatment which includes a proton pump inhibitor and two antibiotics (amoxicillin and clarithromycin) is most commonly recommended [16-18]. However, in recent years the decreased effectiveness of the triple therapy has been reported with eradication rates lower than 80% [19-21]. It is evident from the several studies that the main cause of treatment failure is clarithromycin resistant [22]. However, in developing countries re-infection has also the great contribution in treatment failure and probably more extensive if re-infected with clarithromycin resistant strains.

Mechanism of Antimicrobial Resistance in *H. pylori*

Initially after the introduction of antimicrobials for the treatment it was believed that the evolution of resistance was not likely to be happened. Previously it was assumed that the frequency of mutations resulting resistance in bacteria was negligible [23]. The antibiotic resistance mediated by the mutations in antibiotic targets is by far the most common mechanism in *H. pylori*.

Amoxicillin Resistance

Amoxicillin, a derivative of ampicillin and a beta-lactam antibiotic, was discovered in 1958 and came into medical use in
1972 [24,25]. Penicillin binding proteins (PBPs) are the bacterial peptidoglycan synthesizing enzymes having trans-peptidase activity in the C-terminal region [26]. Beta-lactam antibiotics (amoxicillin) acts by inhibiting the synthesis of cell wall by binding with PBPs and halting its trans-peptidase activity [27]. Several studies have depicted the substitution of one amino acid to another in the PBP1 leading to the resistance to the action of amoxicillin. The substitution of serine (S) at 414 to arginine (R) (S414R) in PBP1 was found to significantly increase the MIC (>0.5-1mg/L) and amoxicillin resistance [28]. Qureshi et al. [29] demonstrated that the substitution of amino acid at position 469 (Y to M), 473 (F to L) and 543 (S to R) can affect the susceptibility of H. pylori to amoxicillin.

**Clarithromycin Resistance**

Clarithromycin is a bacteriostatic antibiotic belonging to the group of macrolide that inhibits the growth of bacteria by inhibiting the protein synthesis. Clarithromycin is one of the essential components of the standard triple therapy [17]. However, its continuous use for the treatment of other infectious diseases such as respiratory tract infections has diminished its efficacy against H. pylori infections [30]. The major mutations implicated in the clarithromycin resistant are A2143G, A2142G and A2142C as depicted by several studies [31-33]. A recent study conducted by Chen at al. in clinical isolates from the United States also revealed the role of these mutations in mediating clarithromycin resistance in majority of the isolates. They reported that the mutations A2143G, A2142G and A2142C mediated the resistance in 82%, 14% and 4% of isolates respectively [32]. In addition to A2143G in 23S rRNA gene, we also confirmed mutations responsible for increased MIC values such as one mutation in infB, a translation initiation factor encoding gene (G160A) and two mutations in rpl22, a ribosomal protein 22 encoding gene (9bp insertion or 3bp deletion) [34].

**Levofloxacin Resistance**

Levofloxacin is a member of the fluoroquinolone antibiotics with a potent and broad-spectrum activity against Gram-negative bacterial pathogens which targets the bacterial DNA gyrase resulting in the impairment of DNA replication [35,36]. The most common mechanism of high level levofloxacin resistance in H. pylori is the point mutation in the DNA gyrase (gyrA and gyrB). The point mutation in gyrA at amino acid 87 and 91 is the most common mutations documented leading to the fluoroquinolone resistance in H. pylori. The common mutations in amino acid are asparagine (N) at position 87 to lysine (L) or tyrosine (Y) (N87L or Y), aspartic acid (D) at 91 to glycine (G) or asparagine (N) or tyrosine (Y) (D91G or N or Y) [37,38]. Recently, we also reported the common mutations in gyrA in levofloxacin resistant strains such as amino acid N at position 87 to lysine (K) or Isoleucine (I) or tyrosine (Y) (N87K or I or Y) and amino acid D at 91 to N or Y or G (D91N or Y or G) [34,39]. The mutation in gyrB is not so common mechanism for the antibiotic resistance. However, a novel mutation at position 463 in gyrB leading to the fluoroquinolone resistance was proposed by Rimbara et al. [40].

**Metronidazole Resistance**

In a recent meta-analysis conducted in Asia-Pacific region, 44% of the H. pylori isolates were reported metronidazole resistance [41]. Metronidazole, a pro-drug which contains a nitro group in its imidazole ring, is activated by reduction of its nitro group which leads to the production of helicoidal DNA-damaging compounds such as nitroso- and hydroxyl amine. The oxygen-insensitive NADPH nitroreductase (RdxA), NADPH-flavin-oxidoreductase (FrxA) and ferredoxin like enzymes (FrxB) performs the reducing activity of metronidazole in H. pylori [42]. Teh et al. [43] detected mutations as the common mechanism for the metronidazole resistant. Out of 37 metronidazole resistant strains insertion or deletion type of mutation was noticed in 33 strains [43]. They also reported that 10.8% of metronidazole resistant strains did not contain any mutations in either rdxA or frxA depicting that mutations in frxB or other mechanism rather in commonly reported may have role. Recently, we reported similar mutations in rdxA and frxA in clinical isolates from Indonesia and Nepal [34,39]. In search for the novel genetic mutations for metronidazole resistance using next generation sequencing we detected the single nucleotide polymorphism in addition to the insertion-deletion in rdxA in metronidazole resistant strains. We suggested that the mutations in frxA are able to increase the metronidazole resistance only when there are mutations in rdxA [44].

**Tetracycline Resistance**

The antibiotic tetracycline is a broad-spectrum antibiotic belonging to the first generation tetracyclines [45]. This antibiotic is a broad-spectrum agent exhibiting bacteriostatic activity against wide range of Gram-positive as well as Gram-negative bacteria. Mutation in the 16S ribosomal subunit at positions 965-967 (AGA) is reported to be responsible for the tetracycline resistance [46,47]. Single and double mutations are associated with low and intermediate MIC values while simultaneous triple mutations are associated with high MIC values [48]. In a study conducted by Wu et al. [49] 54% of tetracycline resistant strains carried single nucleotide substitution such as A965C, A965G, A965T, A967C or A967T and with slightly increased MIC values [49]. Nonaka et al. [48] suggested that the guanine (G) at position 966 plays more important role for the primary site of tetracycline binding and its substitution with other nucleotide results in the high MIC value.

**Role of Bacterial Virulence Factors in Antibiotic Mediated Clearance**

In H. pylori the specific genotype also have been claimed to be involved in the antibiotic mediated bacterial clearance. In several reports, it was found that the strains with vacA s2m2 and cagA-negative status were more resistant to clarithromycin than that of vacA s1m1/vacA s1m2 and cagA-positive status [50]. We previously suggested that the patients infected with more
virulent strains \( \text{vacA} \) s1m1 and \text{cagA}-positive status) show high rate of antibiotic mediated bacterial clearance because of the enhanced inflammatory process and blood flow with increased diffusion of antibiotics to the site of infection [51].

**Conclusion**

Taken together, the above mentioned information suggests that *H. pylori* is one of the bacteria which frequently becomes resistant to common antibiotics by point mutations in the antibiotic target genes. A continuous effort is required to search for the novel mutations and for the identification of their role in antibiotic resistance.

**References**

1. The EUROCOST Study Group (1993) Epidemiology of, and risk factors for, *Helicobacter pylori* infection among 3194 asymptomatic subjects in 17 populations. Gut 34(12): 1672-1676.
2. Rowland M, Daly L, Vaughan M, Higgins A, Bourke B, et al. (2006) Age-specific incidence of Helicobacter pylori. Gastroenterol 130(1): 65-72.
3. Rothenbacher D, Brenner H (2003) Burden of Helicobacter pylori and Helicobacter pylori-related diseases in developed countries: recent developments and future implications. Microbes Infect 5(9): 695-703.
4. Mamishi S, Eshghi H, Mahmoudi S, Bahador A, Hosseinpour Sadeghi R, et al. (2016) Intrafamilial transmission of helicobacter pylori: genotyping of faecal samples. Br J Biomed Sci 73(1): 38-43.
5. Goh KL, Chan WK, Shiota S, Yamaoka Y (2011) Epidemiology of Helicobacter pylori infection and public health implications. Helicobacter 16(1): 1-9.
6. Bui D, Brown HE, Harris RB, Oren E (2016) Serologic evidence for fecal-oral transmission of helicobacter pylori. Am J Trop Med Hyg 94(1): 82-88.
7. Ansari S, Yamaoka Y (2017) Survival of Helicobacter pylori in gastric acidic territory. Helicobacter 22(4): e12386.
8. Campuzano MG (2014) Hematologic manifestations of Helicobacter pylori infection. World J Gastroenterol 20(36): 12818-12838.
9. Ferlay J, Sieri J, Malvezzi M, Tajkari LL, Forman D, et al. (2010) Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136(5): 359-386.
10. McGoll KE (2010) Clinical practice. Helicobacter pylori infection. N Engl J Med 362(17): 1597-1604.
11. Ford AC, Forman D, Hunt RH (2014) Helicobacter pylori eradication therapy to prevent gastric cancer in healthy asymptomatic infected individuals: systematic review and meta-analysis of randomised controlled trials. BMJ 348: g3174.
12. Lopez GS, Puig I, Calvet X (2015) Systematic review and meta-analysis: susceptibility-guided versus empirical antibiotic treatment for Helicobacter pylori infection. J Antimicrob Chemother 70(9): 2447-2455.
13. Vakh N, Megraud F (2007) Eradication therapy for Helicobacter pylori. Gastroenterol 133(3): 985-1001.
14. Boyanova L, Mitov I (2010) Geographic map and evolution of primary Helicobacter pylori resistance to antibacterials. Expert Rev Anti Infect Ther 8(1): 59-70.
15. Megraud F (2004) Helicobacter pylori antibiotic resistance: prevalence, importance, and advances in testing. Gut 53(9): 1374-1384.
16. Wu TS, Hu HM, Kuo FC, Kuo CH (2014) Eradication of Helicobacter pylori infection. J Med Sci 30(4): 167-172.
17. Coelho LG, Maguinilk I, Zaterka S, Parente JM, Moraes-Filho JP, et al. (2013) Third Brazilian consensus on helicobacter pylori. Arq Gastroenterol 150(2): 81-96.
18. Mallortheiner P, Megraud F, Atherton J, Axon AT, Bazzoli F, et al. (2012) Management of Helicobacter pylori infection—the Maastricht IV/Florence Consensus Report. Gut 61(5): 646-664.
19. Graham DY, Fischbach L (2010) Helicobacter pylori treatment in the era of increasing antibiotic resistance. Gut 59(8): 1143-1153.
20. Megraud F (2004) Helicobacter pylori antibiotic resistance: prevalence, importance, and advances in testing. Gut 53(9): 1374-1384.
21. Lee JW, Kim N, Nam RH, Park JH, Choi YJ, et al. (2014) Geno Type HelicoDR test in the determination of antimicrobial resistance of Helicobacter pylori in Korea. Scand J Gastroenterol 49(1): 1058-1067.
22. Rimbara E, Noguchi N, Kijima H, Yamaguchi T, Kawai T, et al. (2007) Mutation in the 23S rRNA gene of clarithromycin-resistant Helicobacter pylori from Japan. Int J Antimicrob Agents 30(3): 250-255.
23. Davies J (1994) Inactivation of antibiotics and the dissemination of resistance genes. Science 264(517): 375-382.
24. Janos F, Robin GC (2006) Analogue-based Drug Discovery.
25. Roy J (2012) An introduction to pharmaceutical sciences production, chemistry, techniques and technology. Cambridge: Woodhead Pub.
26. Harris AG, Hazell SL, Netting AG (2000) Use of digoxigenin-labelled ampicillin in the identification of penicillin-binding proteins in Helicobacter pylori. J Antimicrob Chemother 45(5): 591-598.
27. Megraud F, Lehours P (2007) Helicobacter pylori detection and antimicrobial susceptibility testing. Clin Microbiol Rev 20(2): 280-322.
28. Gerrits MM, Godoy AP, Kuipers EJ, Ribeiro ML, Stoof J, et al. (2006) Multiple mutations in or adjacent to the conserved penicillin-binding protein motifs of the penicillin-binding protein IA confer amoxicillin resistance to Helicobacter pylori. Helicobacter 11(3): 181-187.
29. Qureshi NN, Morikis D, Schiller NL (2011) Contribution of specific amino acid changes in penicillin binding protein 1 to amoxicillin resistance in clinical Helicobacter pylori isolates. Antimicrobial Agents Chemotherapy 55(1): 101-109.
30. Taniike I, Goshi S, Tamura Y, Makishita-Saito N, Matsumori N, et al. (2002) Emergence of clarithromycin-resistant Helicobacter pylori (CRHP) with a high prevalence in children compared with their parents. Helicobacter 7(5): 297-305.
31. Megraud FH (2004) H pylori antibiotic resistance: prevalence, importance, and advances in testing. Gut 53(9): 1374-1384.
32. Chen D, Cunningham SA, Cole NC, Kohner PC, Mandrekas JN, et al. (2017) Phenotypic and molecular antimicrobial susceptibility of Helicobacter pylori. Antimicrobial Agents Chemotherapy 61(4).
33. Aguilema-Correa JJ, Urruzuno P, Barrio J, Martinez MJ, Aguado S, et al. (2017) Detection of Helicobacter pylori and the genotypes of resistance to clarithromycin and the heterogeneous genotype to this antibiotic in biopsies obtained from symptomatic children. Diagn Microbiol Infect Dis 87(2): 150-153.
34. Binh TT, Shiota S, Suzuki R, Matsuda M, Trang TTH, et al. (2014) Phenotypic and molecular antimicrobial susceptibility of Helicobacter pylori in Korea. Scand J Gastroenterol 49(1): 1058-1067.
35. Lesher GY, et al. (1962) 1,8-Naphthyridine derivatives-A new class of antibacterial agents. J Med Chem 5: 198-205.
36. Drlica K (2009) Quinolones: action and resistance updated. Curr Top Med Chem 9(11): 981-998.
37. Suzuki H, Nishizawa T, Munoaka H, Hibi T (2009) Sitafloxacin and garenoxacin may overcome the antibiotic resistance of Helicobacter
38. Miftahussurur M, Shrestha PK, Subsomwong P, Sharma RP, Yamaoka Y, et al. (2016) Emerging Helicobacter pylori levofloxacin resistance and novel genetic mutation in Nepal. BMC Microbiol 16(1): 256.

39. Rimbara E, Noguchi N, Kawai T, Sasatsu M (2012) Fluoroquinolone resistance in Helicobacter pylori: role of mutations at position 87 and 91 of gyrA on the level of resistance and identification of a resistance conferring mutation in gyrB. Helicobacter 17(1): 36-42.

40. Kuo YT, Liou JM, El-Omar EM, Wu JY, Leow AHR, et al. (2017) Primary antibiotic resistance in Helicobacter pylori in the Asia-Pacific region: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol 2(10): 707-715.

41. Francesco VD, Zullo A, Hassan C, Giorgio F, Rosania R, et al. (2011) Mechanisms of Helicobacter pylori antibiotic resistance: an updated appraisal. World J Gastrointest Pathophysiol 3(3): 35-41.

42. Teh X, Khooraei Y, Lee WC, Leow AHR, Loke MF, et al. (2014) Functional and molecular surveillance of Helicobacter pylori antibiotic resistance in Kuala Lumpur. PLoS ONE 9(7): e101481.

43. Binh TT, Suzuki R, Trang TTH, Kwon DH, Yamaoka Y, et al. (2015) Search for novel candidate mutations for metronidazole resistance in Helicobacter pylori using next-generation sequencing. Antimicrob Agents Chemother 59(4): 2343-2348.

44. Fuoco D (2012) Classification framework and chemical biology of tetracycline-structure-based drugs. Antibiotics 1(1): 1-13.