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Who's Winning the War? Molecular Mechanisms of Antibiotic Resistance in *Helicobacter pylori*

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Abstract

The ability of clinicians to wage an effective war against many bacterial infections is increasingly being hampered by skyrocketing rates of antibiotic resistance. Indeed, antibiotic resistance is a significant problem for treatment of diseases caused by virtually all known infectious bacteria. The gastric pathogen *Helicobacter pylori* is no exception to this rule. With more than 50% of the world's population infected, *H. pylori* exacts a tremendous medical burden and represents an interesting paradigm for cancer development; it is the only bacterium that is currently recognized as a carcinogen. It is now firmly established that *H. pylori* infection is associated with diseases such as gastritis, peptic and duodenal ulceration and two forms of gastric cancer, gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. With such a large percentage of the population infected, increasing rates of antibiotic resistance are particularly vexing for a treatment regime that is already fairly complicated; treatment consists of two antibiotics and a proton pump inhibitor. To date, resistance has been found to all primary and secondary lines of antibiotic treatment as well as to drugs used for rescue therapy.

The war between antibiotics and bacteria

The knowledge that bacteria were susceptible to antibiotics had its genesis in 1877 with the first report by Pasteur and Joubert that a bacterium produced a toxic substance that killed other bacteria (cited in [1]). Fifty-two years later, penicillin was identified in 1929 as an antimicrobial by Fleming [2]. After it was shown that penicillin was able to prevent a lethal infection of streptococcus in mice [3] and to treat human disease [4, 5], it was thought that antibiotics would easily eliminate infectious disease. However, to everyone's surprise, bacteria are strategic fighters, and have adapted to become resistant to a multitude of antibiotics used to treat infection. Because of this, it is no exaggeration to say that, on some fronts, bacteria are on the verge of winning the war.

Broadly defined, antibiotics are either natural or synthetic products that kill (bactericidal) or inhibit growth (bacteriostatic) of bacteria. Antibiotics must be selectively toxic for the microbe, and current drug classes target four key aspects of bacterial growth and physiology. The targets include bacterial cell wall synthesis, foliate coenzyme synthesis, protein synthesis, and nucleic acid (DNA or RNA) synthesis [6]. Bacterial antibiotic resistance mechanisms can similarly be broken into five major classes. These include alteration of the antibiotic target, enzymatic destruction of the antibiotic, enzymatic modification of the

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antibiotic, decreased permeability of the antibiotic, and increased efflux of the antibiotic from the bacterial cell (Table 1) [6].

Introduction of the enemy: *Helicobacter pylori*

Helicobacter pylori was first isolated from patients suffering from chronic gastritis in 1982 [7]. *H. pylori* is a Gram-negative, spiral shaped bacterium that colonizes the arguably inhospitable niche of the stomach, and persists for the lifetime of the host, if untreated [8]. Additionally, *H. pylori* causes a wide range of diseases. This organism is associated with gastritis, 90% of all duodenal ulcers, 75% of all gastric ulcers [9], and two forms of stomach cancer, adenocarcinoma and MALT lymphoma [9–12]. Though infection rates show wide geographic distribution, this strategic bacterium chronically colonizes over 50% of the world's population [13]. The high incidence of *H. pylori* infection likely contributes to the fact that gastric cancer mortality ranks second among all cancer deaths worldwide [14]. Due to the causal relationship between *H. pylori* and gastric malignancies, the World Health Organization classified *H. pylori* as a Class I carcinogen in 1994 [15]. Currently, *H. pylori* is the only bacterium to have achieved this perilous distinction.

During the relatively short period of time that we have known about *H. pylori*, there have been many different treatment regimens developed (reviewed in [16]. In fact, in 1994 there was a consensus from the National Institute of Health (USA) [17], followed two years later by the Maastricht Consensus from the European *Helicobacter* Study Group (Netherlands) [18], which established treatment recommendations to treat *H. pylori* infection. Given the increase in incidence of antibiotic resistance, the Maastricht Consensus report was updated in 2000 and again in 2005 to increase the effectiveness of treatment regimens against *H. pylori* [19, 20].

The current recommendation for first line therapy in locations where clarithromycin resistance is low, is a proton pump inhibitor, clarithromycin, and either metronidazole (first choice) or amoxicillin (second choice) for 14 days [20]. Additionally, these triple therapy regimens can be supplemented by the addition of bismuth in geographical areas where antibiotic resistance is high, though this combination is typically recommended as a second line therapy [20]. Moreover, since bismuth is not available in many countries, a combination of a proton pump inhibitor, metronidazole, and either amoxicillin or tetracycline is sometimes recommended [20].

Primary and secondary therapies are not always successful at eradicating *H. pylori*; therefore, there are many alternative drugs that are proposed for rescue therapy. These include fluoroquinolones (such as levofloxacin), rifamycins (such as rifabutin and rifampicin), nitrofurans (such as furazolidone) and other members (such as doxycycline) within families that are already used to treat *H. pylori* infection (reviewed in [16, 21]. Of note, resistance has been found to all utilized primary and secondary antibiotics, as well as, to many of the antimicrobials used for rescue therapy. This fact suggests that therapy success rates will continue to decline, and indicates that a detailed understanding of antibiotic resistance mechanisms may facilitate development of novel therapeutics. As such, the molecular mechanisms of *H. pylori* antimicrobial resistance are discussed in detail in this review (Table 2 and Figure 1).

First Line Therapy and Resistance

Macrolides (primarily clarithromycin)—Clarithromycin is one of the first line therapy antibiotics used against *H. pylori* and is part of a class of broad spectrum antibiotics called macrolides [20]. Macrolides function to prevent protein translation. Specifically, these

antibiotics interact with the bacterial ribosome and promote the premature release of peptidyl-tRNA from the acceptor site [22, 23].

Macrolide resistant *H. pylori* strains have been shown to be selected for during the course of treatment [24]. Moreover, clarithromycin resistance has been pinpointed to nucleotide mutations, the majority of which are single nucleotide mutations, in one of the two macrolide binding domains of the 23S rRNA [25, 26]. Most significant mutations are contained in Domain V of the 23S rRNA. In fact, this mechanism of clarithromycin resistance in *H. pylori* was first described in 1996 by Versalovic *et al.* Since the nomenclature for these mutations is confusing due to variation in the length of the 23S rRNA gene among strains, for this review we will employ the nomenclature for the 23S rRNA gene published by Taylor *et al.* [27]. At first, single nucleotide substitutions of an adenine to guanine at nucleotide positions 2142 (A2142G) and 2143 (A2143G), of the 23S rRNA were discovered [22]. Subsequently these same mutations were found in several other clarithromycin resistant strains [22, 24, 27–32]. Additional evidence that mutations within this domain are important for macrolide resistance in *H. pylori* came with the discovery that a transversion of an adenine to cytosine at position 2142 (A2142C) also conferred clarithromycin resistance [33]. In some studies as many as 91.4% of clarithromycin resistant strains contain the A2142G or the A2143G mutation [30]. Moreover, these are the predominant mutations found in Brazil [34], France [35], and Spain [36].

Other mutations within domain V of the 23S rRNA that have been identified to confer macrolide resistance include T2182C [37], A2144T [38], G2223A, C2244T, and T2288C [39], though the ability of the T2182C mutation to confer macrolide resistance remains in question, due to the fact that this mutation has been found in both resistant and sensitive strains [40]. The double mutation of A2115G and G2141A has also been demonstrated to confer resistance [29, 41].

Other important clarithromycin resistance mutations lay outside of domain V of the 23S rRNA. One such mutation, T2717C, results in low level clarithromycin resistance [42]. Additionally, other clarithromycin resistant *H. pylori* strains contain no mutations in the 23S rRNA [28, 42]. Though the exact mechanism remains undefined, this fact indicates another means of obtaining macrolide resistance. In other bacteria, macrolide resistance can occur through methylation of a specific adenine on the 23S rRNA [25, 43, 44]. This is achieved through the presence of rRNA methylases, termed *erm* genes (erythromycin resistance methylase) [43]. Despite the presence of clarithromycin resistance that is not associated with mutations of the 23S rRNA, scientists have failed to clone a single erythromycin resistance determinant from any *H. pylori* resistant isolate tested, and have failed to find genes with homology to any previously reported *erm* genes [29]. This has left the nature of this resistance in question. Interestingly, *H. pylori* has the ability to remove a different macrolide, erythromycin, via the HefABC efflux pump; however, *H. pylori* cannot efflux clarithromycin through this pump [45]. Based on the studies described above, it is clear that continued treatment of *H. pylori* with clarithromycin will likely lead to increasing rates of resistance, and may lead to resistance to multiple macrolides due to cross-resistance [29].

Beta-lactams (amoxicillin)— β -lactams are one of the best weapons clinicians have against *H. pylori*. The β -lactam antibiotics are subdivided into 5 groups. These include penicillins, cephalosporins, carbapenems, monobactams, and clavams [6]. All β -lactam antibiotics inhibit synthesis of the peptidoglycan layer of the bacterial cell wall. They do so by targeting penicillin binding proteins (PBPs) on the cytoplasmic membrane [46]. These PBPs are enzymes that carry out carboxypeptidation and transpeptidation, which are the terminal steps of peptidoglycan biosynthesis [46–48]. Broadly speaking, in other bacteria resistance to β -lactams arises by decreased membrane permeability of the drugs, increased

efflux of the drug from the bacterial cell, modification of the PBPs that diminish the affinity of the drug for the protein, and the presence of β -lactamases that inactivate the antibiotic by hydrolyzing its ring structure [6].

Amoxicillin is a semi-synthetic penicillin that is currently the only β -lactam used to treat *H. pylori* infection. While initial treatment with amoxicillin suggested that it was very effective, amoxicillin resistance was documented in 1998, when the Hardenberg strain was isolated in Holland from an 82 year old dyspeptic patient [49]. Currently, amoxicillin resistance rates vary from as low as 0% to as high as 59% [50–53].

H. pylori contains nine putative penicillin binding proteins [54–56]. Of these, mutations of PBP1 [57–62] and PBP4 (also known as PBDP) [54, 55] have been shown to impact amoxicillin resistance. In terms of PBP4, a decreased amount of this protein was demonstrated to confer low level resistance to amoxicillin [55]. In fact, other resistant strains were confirmed to have no detectable PBP4 [54, 55].

In contrast, amino acid mutations in PBP1 can result in resistance due to decreased affinity for amoxicillin [57]. Studies have shown that most amino acid substitutions that result in resistance occur in the carboxy terminus of PBP1 in the penicillin binding domain [63]. For instance, PBP1 from the Hardenberg strain contains a single amino acid substitution of serine to arginine at position 414 (Ser414Arg) [58, 63]. Interestingly, Kwon *et al.* deduced that β -lactam resistance was acquired with multidrug resistance, and that 10 different amino acid substitutions in the PBP1 penicillin binding domain could confer resistance: Glu406Ala, Ser417Thr, Met515Ile, Asp535Asn, Ser543Arg, Thr556Ser, Asn562Tyr, Lys648Gln, Arg649Lys, and Arg656Pro [59]. Two of the previously described amino acid mutations (Thr556Ser [63, 64] and Asn562Tyr [63]) were later confirmed, and additional amino acid mutations conferring resistance were identified; Ala369Thr, Val374Leu, Leu423Phe, Thr593Ala, and Gly595Ser [63]. Additionally, an *in vitro* study found that Thr438Met mutation is sufficient to cause resistance [60]. Another study showed that a nonsense mutation in the 3' end was enough to confer resistance [64].

While mutations in PBP2 (*ftsI*) and PBP3 (*pbp2*) alone are enough to confer resistance to other β -lactams (ceftriaxone and ceftazidime), no mutations within either of these genes individually conferred resistance to amoxicillin [65]. However, combined mutations in PBP1 (Ser414Arg, Leu423Phe, Asn562Tyr, and Thr593Ala) and PBP3 (Ala499Val and Glu536Lys) yielded a higher level of resistance to amoxicillin [65]. Interestingly, even though mutations in PBP2 alone or in combination with mutations in PBP1 did not yield or increase amoxicillin resistance, respectively, the combination of mutations in PBP2 (Ala296Val, Asn/Ser494His, Ala/Val541Met, and Glu572Gly) worked synergistically with the aforementioned mutations found in PBP1 and PBP3 to confer an even greater level of amoxicillin resistance [65].

In addition to decreased amoxicillin binding [57], resistance can also result from mutations that decrease membrane permeability of the drug [47, 59, 61]. Mutations in two different outer membrane proteins have been proven to be sufficient to cause amoxicillin resistance [60]. These mutations include changes in amino acids 116–201 of *hopB* or a stop codon at amino acid 211 of *hopC* [60].

Nitroimidazoles, primarily metronidazole

Nitroimidazoles are synthesized as inactive prodrugs and require reduction inside the bacteria by specific non-human reductases to become active [66, 67]. In their active forms, nitroimidazoles produce toxic intermediates that result in DNA damage that kills the bacteria [66]. Metronidazole is a nitroimidazole that was efficiently used to treat *H. pylori*

infection. However, *H. pylori* has adapted to be able to strategically escape the effectiveness of metronidazole treatment. Metronidazole resistance rates vary from 29–52% [68, 69] in some regions, and are as high as 100% in certain places [70].

The mechanism for metronidazole resistance in *H. pylori* is probably the most studied and most controversial topic concerning antibiotic resistance in the *H. pylori* field. Early studies showed that resistant strains accumulated metronidazole at a slower rate and to a lesser extent than sensitive strains, suggesting a role for transporters or efflux systems in resistance [71]. Other work suggested that mutations in *recA* might be responsible for resistance [72], but later studies found no evidence that mutations in *recA* lead to metronidazole resistance [73].

The first real breakthrough in understanding metronidazole resistance came with the discovery that resistant bacteria reduce other 5-nitroimidazole compounds more slowly than sensitive strains [74]. This indicated that resistance is a product of lack of reduction of the prodrugs [74]. Goodwin *et al.* showed that metronidazole's toxicity depends on its reduction by the oxygen-insensitive, nicotinamide adenine dinucleotide phosphate (NADPH) nitroreductase (*rdxA*), and that resistance arises from mutations that inactivate *rdxA* [75]. Specifically, a nonsense mutation caused the truncation of 14 amino acids, and other point mutations (both nonsense and missense) were found elsewhere in the *rdxA* gene [75].

Many other studies have identified specific mutations in *rdxA* that result in metronidazole resistance [73, 76–78]. The majority of these mutations result in a truncated RdxA protein [79–81]. Other resistant isolates have been identified that encode full length RdxA, but contain amino acid substitutions (Arg16His, Ala80Thr, Ala118Ser, Gln197Lys, Val204Ile [80], Tyr46His, Pro51Leu, Ala67Val [79], and Cys19Tyr [77]).

While inactivation of *rdxA* clearly causes metronidazole resistance, the association of specific amino acid mutations with resistance remains controversial, since very few studies made specific *rdxA* amino acid substitutions and then tested for resistance. Instead, resistant strains are often identified, and then *rdxA* is simply sequenced to identify changes and any differences are ascribed to impart metronidazole resistance, without distinguishing resistance-associated nucleotide mutations from natural genetic diversity. However, newer studies show that the majority of *rdxA* sequences were identical between susceptible and resistant strains, leading the authors to suggest that inactivation of RdxA is sufficient but not essential to obtain metronidazole resistance [78, 82]. This evidence, coupled with the fact that some resistant strains do not have mutations within the *rdxA* gene [76, 79, 83], suggests that other resistance mechanisms exist in *H. pylori*.

Kwon *et al.* were the first to discover that mutations in the NADPH flavin oxidoreductase gene (*frxA*) conferred metronidazole resistance [84]. Deletion mutations resulting in truncations of the *frxA* gene confer metronidazole resistance [84, 85], and missense mutations in *frxA* (Cys161Tyr, Arg206His, and a double mutation of Trp137Arg and Glu164Gly), without specific changes in *rdxA*, confer low level resistance [85]. High metronidazole resistance is conferred when both the *frxA* and *rdxA* genes are prematurely truncated, indicating a synergistic effect on resistance [77, 83–85]. In contrast to these studies, others have found that *frxA* inactivation does not significantly change susceptibility to metronidazole [86]. Also, the majority of resistant strains with mutations in *frxA* also have mutations in *rdxA* [87], and truncations of *frxA* have been identified in metronidazole sensitive *H. pylori* isolates [88]. These facts, combined with work that suggested that inactivation of *frxA* in a strain with a functional *rdxA* only slowed the bactericidal effects of metronidazole [87], has led some to suggest that a *frxA* mutation alone may not be enough to impart resistance [89].

Another controversy in the metronidazole field lies in the debate as to whether other genes besides *rdxA* and *frxA* may have a role in metronidazole resistance. Some metronidazole resistant isolates have functional *rdxA* and *frxA* with no identified mutations, indicating that there are other mechanisms leading to metronidazole resistance [90]. One area of study has focused on the role of various *Helicobacter pylori* reductases in metronidazole resistance. It was found that disruption of the *fdxB* (ferredoxin-like protein) nitroreductase resulted in an increased level of metronidazole resistance in strains with an inactivated *rdxA* [83]. Other reductases, such as alkyl hydroperoxide reductase, AhpC [91], have been suggested to play a role in resistance since the expression levels of various isoforms of *ahpC* are increased when resistant strains are grown in the presence of metronidazole [92]. While thioredoxin reductase has been found to reduce metronidazole in at least one other organism [93], its role in metronidazole resistance in *H. pylori* is still unknown. However, an *in vitro* study showed that thioredoxin protein 1, *trxAl*, can be an electron donor for AhpC [94]. Finally, there is also evidence that an efflux pump (TolC) is responsible for metronidazole resistance. When two of four TolC homologs (HP0605 and HP0971) are mutated, it leads to an increase in susceptibility to metronidazole even though single-knockout mutants are still resistant [95]. Taken together, it is clear that *rdxA* and *frxA* mutations confer metronidazole resistance. However, the role of other genes and mutations require more thorough characterization.

Second Line Therapies and Resistance

Tetracyclines—Tetracyclines are often used as a second line therapy when *H. pylori* infections are not cured by the first line drug regimen. The tetracyclines include tetracycline, chlortetracycline, oxytetracycline, doxycycline, and tigilicycline [6]. Tetracyclines function by inhibiting bacterial protein synthesis. They do this by binding to the 30S ribosomal subunit and blocking the attachment of a new aminoacyl-tRNA to the ribosomal acceptor site [6, 96–98]. This effectively stops synthesis of bacterial peptides. However, since the interaction between the ribosome and the tetracycline is reversible, the antibiotic has a bacteriostatic effect [96, 97].

As with other drugs, resistance patterns to tetracyclines vary by geographic distribution: resistance rates are fairly low (0–7% in some areas) [99, 100], but higher in others (as high as 59% in China) [101]. Tetracycline resistance in *H. pylori* was first observed in 1996 [102], and primarily occurs through alteration of nucleotides within the primary tetracycline binding site [103]. For instance, a triple mutation in the 16S rRNA gene at nucleotides 926–928, from AGA to TTC, has been shown to confer high level tetracycline resistance and is found worldwide [103–107]. Additionally, single and double mutations in this region weaken the interaction of 16S RNA with tetracycline, and result in moderate resistance to tetracycline [99, 106, 108, 109].

While changes in the 16S rRNA sequence are the primary mechanism of tetracycline resistance, there are reports of weakly resistant *H. pylori* strains with no mutations in their 16S rRNA [108, 109]. Moreover, some of these tetracycline resistant strains demonstrate normal ribosome-tetracycline binding [108]. While the mechanism of resistances in these strains is unclear, some possible explanations include the presence of efflux pumps or mutations in various porin genes [109]. Putative efflux systems have been identified in *H. pylori* [110]. Although no observable tetracycline efflux activity was initially found [111], a later study found that the efflux pump, HefABC, actually does show efflux activity for tetracycline [45]. Additionally, mutations within this pump results in an increase in susceptibility to tetracycline [45].

Rescue/Salvage Therapy and Resistance

Fluoroquinolones—Some of the newer weapons used in the war against *H. pylori* are the fluoroquinolones. Fluoroquinolones include ciprofloxacin, gatifloxacin, sitafloxacin, moxifloxacin, temafloxacin, and levofloxacin. Currently, these drugs are used as rescue or salvage therapy, when both first and second line therapies have failed to eradicate infection. Fluoroquinolones target topoisomerase II (gyrase) or topoisomerase IV (a gyrase homologue) activity of bacterial cells [112]. Bacterial gyrases act by making cuts in DNA, thus allowing the nucleic acid strand to be in a relaxed orientation, where it is available for replication, recombination, and transcription [6]. Resistance mutations to ciprofloxacin, in other bacteria has been found in amino acids 67–106 of the gyraseA subunit [113]. Thus, this region has been deemed the “quinolone resistance determining region,” or the QRDR [114].

Fluoroquinolone resistance rates vary geographically to as low as 13.8% (Lisbon) [115] to between 21.5% and 33.8 % (depending on the fluoroquinolone) in Korea [116]. As with other bacteria, most quinolone resistance mutations in *H. pylori* are found within the QRDR. The first amino acid mutations identified were Asn87Lys, Ala88Val, Asp91Gly, Asp91Asn, Asp91Tyr [116–118], and a double mutation at amino acid Asp91 to a Gly, Asn, or Tyr combined with either a Ala97Val or Ala97Asn substitution [116, 118]. The Ala97Val mutation has subsequently been shown not to be associated with resistance when found alone [119]. Other double mutations identified as being important in fluoroquinolone resistance are Ala84Pro/Ala88Val and Ser83Ala/Asn87Lys [118]. Miyachi *et al.* found three new mutations causing levofloxacin resistance to be Asn87Ile, Asn87Tyr, and a double mutation of Asn87His and Asp91Gly [120]. Indeed, many studies have confirmed that the major mutation causing fluoroquinolone resistance occurs in *gyrA* at position 91 [116, 119, 121] or 87 [118, 120]: studies have shown that 95.7% of gatifloxacin resistant strains [121] and 83.3% of levofloxacin resistant strains [120] contains mutations at these residues.

Currently, only a single resistant strain has been identified that does not contain a mutation within the QRDR [118]. This discovery suggests that there are additional mechanisms of fluoroquinolone resistance in *H. pylori*. Common mechanisms of resistance found in other bacteria, such as efflux pumps and topoisomerase IV, appear not to apply here, since there is evidence that efflux pumps are not a strategy for antimicrobial resistance to fluoroquinolones in *H. pylori* [45, 111] and genes such as *parC* or *parE* are not found in the *H. pylori* genome [119, 122, 123]. Several studies have also looked at *gyrB*, but no mutations resulting in resistance were isolated [119–121]. Therefore, currently the alternative mechanism of resistance remains unknown, and further studies need to be completed to have a more inclusive understanding of fluoroquinolone resistance.

Rifamycins—Rifamycins, which consists of rifampicin and rifabutin, are used for rescue therapy. These drugs are bactericidal, due to the irreversible blockage of the DNA-dependent RNA polymerase [124]. While there are no prevalence studies for resistance to rifamycins, resistance is possible by mutation within the *rpoB* gene, which encodes the β -subunit of RNA polymerase [125]. Several different amino acids substitutions have been shown to confer rifamycin resistance: Leu525Pro, Gln527Lys [126], Gln527Arg, Asp530Val [126, 127], Asp530Asn [126], His540Tyr [126, 127], His540Asn [126], Ser545Leu [126, 127], Ile586Asn, and Ile586Leu [126]. Not surprisingly, these regions are also important for rifamycin resistance in *E. coli* and mycobacteria [126]. Other amino acids substitutions within *rpoB* that have been shown to be important for rifamycin resistance include Val149Phe [128, 129] and Arg701His [129].

Nitrofurans—Nitrofurans are prodrugs that become active when the nitro group is reduced by an oxygen-insensitive nitroreductase. This activation results in the production of electrophilic intermediates that cause DNA damage and attack bacterial ribosomal proteins, thus blocking protein synthesis and causing cell death [130–132]. Nitrofurans consist of furazolidone and nitrofurantoin. Prevalence of nitrofuran resistance has been shown to be between 1.6% [133] and 4% [51]. This low resistance rate could be attributable to the fact that nitrofurans are not widely used.

While the mechanism of action of these drugs are similar to 5-nitroimidazoles, resistance is not acquired through the same mechanisms. While 5-nitroimidazole (such as metronidazole) resistance arises from mutations within *rdxA* and *frxA*, knockouts of these genes did not produce resistance to furazolidone or nitrofurantoin [133]. This suggests that the nitrofurans could be used as an alternative to metronidazole [133].

It has been suggested that activation of the nitrofurans requires two reduction steps such as is the case in *E. coli* [131, 133]. Evidence for two reduction steps includes: the fact that *in vitro* serial passage does not produce any nitrofuran resistant strains [134] and that despite the fact that FrxA has the ability to reduce nitrofurans [135], knockouts of *frxA* do not confer nitrofuran resistance [133]. Additional nitroreductases suggested as having a role in nitrofuran resistance in *H. pylori* are pyruvate:flavodoxin oxidoreductase (PorCDAB) [133, 135] and 2-oxoglutarate oxidoreductase (OorDABC) [133]. When these genes are mutated, a low-level resistance is conferred to nitrofurans as well as to metronidazole [133]. If resistance is acquired in a two step mechanism, these antibiotics may be the next good weapon physicians have against *H. pylori*.

Alternative treatments methods

Characteristics of *H. pylori*, such as high colonization rates, longevity of infection, severity of associated diseases, and the ability to become resistant to varying antibiotics with diverse mechanisms of action, has led to investigation into alternative treatment methods. These alternatives include modifying diet or vitamin intake, developing a vaccine for *H. pylori*, and modifying existing treatment regimes. Modifications to diet or vitamin intake includes the addition of vitamin C supplements, which may reduce risk of gastric maladies [136–138] and *H. pylori*'s ability to colonize [139, 140], and prostaglandins (either taken directly or obtained through polyunsaturated fatty acids), which protect the gastric mucosa from damage [141–143].

Arguably the best option would be the production of a *H. pylori* vaccine, and several possible vaccine candidates are being researched [144–146]. Vaccine components vary and include killed *H. pylori* whole cell extracts [147], heat shock proteins [148], flagellar antigens [144], adhesion antigens [149], lipopolysaccharide antigens [150], neutrophil activating protein [151], and urease [152]. Unfortunately many of these are a long way from human trials [144–146], and the inactivated whole cell extract were proven ineffective in a human volunteer study [153].

In lieu of an affective vaccine, many new drugs have shown promise against *H. pylori*. For instance, other prodrugs use different primary reductases than metronidazole, providing an alternative to a drug that is ineffective in many areas [135]. Newer fluoroquinolones (sitafloxacin, HSR-903, gatifloxacin, Bay 12-8039, and trovafloxacin) [154–156] and new rifamycins (KRM-1657 and KRM-1648) [157] show *in vitro* activity against *H. pylori* at low concentrations, suggesting that they may be affective *in vivo*.

Modifications to current therapeutic regimes include the addition of lactoferrin, which has been proven to improve eradication rates when used with certain triple therapies [158] and lactobacillus, which has been proven to lower side effects of some antibiotics thus, potentially increasing patient compliance [159, 160]. A final possible modification to current therapeutic regimes is the addition of a metal, such as bismuth or cobalt II, to the treatment regimes. Bismuth compounds prolong antibiotic use by reducing resistance rates as well as producing a synergistic effect with several drugs [161, 162]. Cobalt II shows particular promise, as it is effective at 100 times lower concentrations than bismuth [163]. Along these lines, since two enzymes essential for colonization, hydrogenase and urease [164–167], require nickel for maturation [168–174] and there is no clear biochemical function for nickel in the human host [175], theoretically nickel chelation could be used as an effective antimicrobial agent.

H. pylori has evolved into a highly antimicrobial resistant pathogen, obtaining resistance to almost all first, second, and rescue therapy antibiotics. Scientists have been able to identify many of the molecular mechanisms that confer resistance (Table 2 and Figure 1). However, there are still some unknown resistance mechanisms to identify. While currently it may seem as if *H. pylori* is winning the war against antibiotics, hopefully new treatment options will improve eradication rate. Overall, it is clear that novel strategies, such as inhibitors of virulence factors [176], and drug development will be required to produce the tools to allow physicians to yet win the war against this strategic pathogen.

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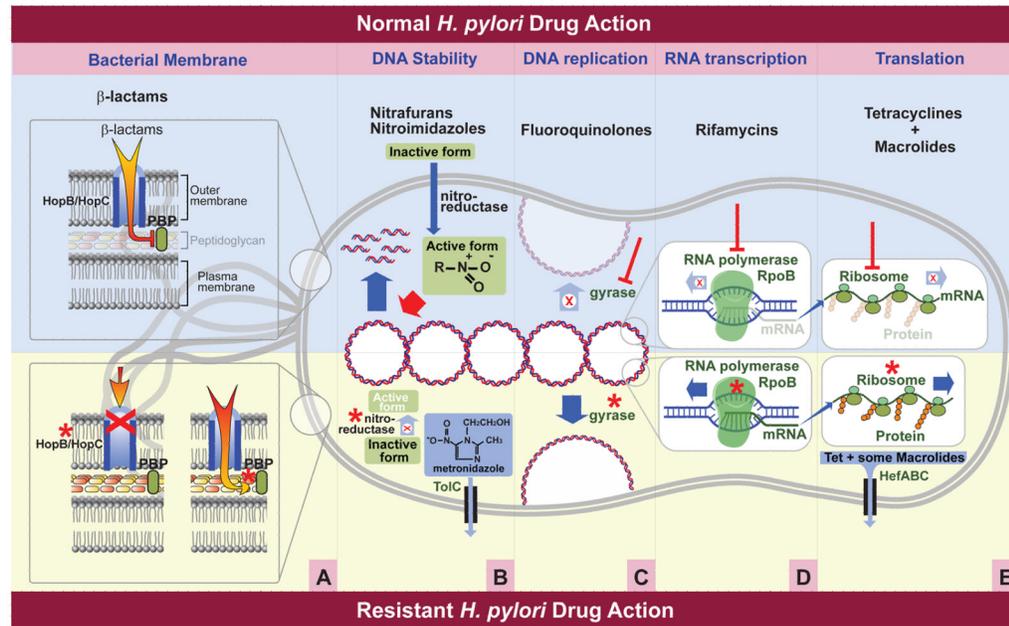


Figure 1.

Cellular components targeted by commonly used antibiotics and mechanisms of resistance utilized by *H. pylori*. The upper portion of the figure depicts normal drug interactions, while the lower portion depicts resistance mechanisms currently identified in *H. pylori*. * Denotes a mutation in the specified gene. A. β -lactams prevent the completion of the peptidoglycan layer of *H. pylori* through their interaction with penicillin binding proteins (PBP). Resistant bacteria contain either a mutated PBP, which prevents interaction with the β -lactams, or mutations in *hopB* or *hopC*, which decrease accumulation of the β -lactam within the bacterial cell. B. Nitrofurans and nitroimidazoles act in a similar manner against bacterial DNA. Both pro-drugs enter the cell and must be reduced by nitroreductases to become active. The activated form leads to formation of radicals that damage DNA. In resistant bacteria one or more of these nitroreductases are inactivated. The existence of a TolC efflux pump has also been identified as a mechanism of resistance to nitroimidazoles. C. Fluoroquinolones act upon gyrases, which are enzymes responsible for the conversion of DNA into a relaxed state required for DNA replication. Bacteria containing mutations in these gyrases are resistant to Fluoroquinolones. D. Rifamycins act by blocking a subunit of the DNA dependent RNA polymerase thereby terminating the production of mRNA. Resistant bacteria contain mutations within this subunit, which is encoded by the *rpoB* gene. E. Tetracyclines and macrolides both prevent the completion of translation, thereby preventing protein production. Mutations within specific ribosomal subunits cause *H. pylori* to be resistant to these drugs. Also, the HefABC efflux pump has been shown to remove tetracycline and some macrolides from the bacterial cell.

Mechanism of resistance to each identified drug family as found in a typical bacterium and the currently identified mechanisms of resistance found in *H. pylori*.

Table 1

| Antibiotic | Antibiotic Target | Typical Mechanisms of Resistance | <i>H. pylori</i> 's Mechanism of Resistance |
|------------------|--|--|---|
| Macrolides | 23S rRNA | Mutation of target or post transcriptional modification of target | Mutation of target |
| β -Lactams | PBP | Mutation of target, decreased permeability, increased efflux, β -lactamase | Mutation of target, decreased permeability |
| Nitroimidazoles | DNA | Mutation of bacterial nitroreductases, increased efflux | Mutation of bacterial nitroreductases, increased efflux |
| Tetracyclines | 30S ribosomal subunit | Mutation of target, increased efflux, mutation of ribosomal protection proteins | Mutation of target, increased efflux |
| Fluoroquinolones | topoisomerase II (gyrase) or topoisomerases IV | Mutation of <i>gyrA</i> or topoisomerase IV, increased efflux | Mutation of <i>gyrA</i> |
| Rifamycins | DNA-dependent RNA polymerase | Mutation of target | Mutation of target |
| Nitrofurans | DNA | Mutation of multiple bacterial nitroreductases | Mutation of multiple bacterial nitroreductases |

Table 2Specific Antibiotic Resistance Mutations found in *H. pylori*.

| Antibiotic Class | Mechanism of resistance | Mutation | Reference |
|---|--|--------------------------------------|------------------|
| Macrolides | Mutation of domain V of the 23S rRNA | A2142G | [22, 24, 27– 32] |
| | | A2142C | [33] |
| | | A2143G | [22, 24, 27– 32] |
| | | A2144T | [38] |
| | | T2182C | [37] |
| | | G2223A | [39] |
| | | C2244T | [39] |
| | | T2288C | [39] |
| | | Double mutation of A2115G and G2141A | [29, 41] |
| | T2289C* | [39] | |
| Mutation outside domain V of the 23S rRNA | T2717C | [42] | |
| Unknown | Unknown | [28, 42] | |
| β -Lactams | Mutation of penicillin/PBP4 complex | Unknown | [54, 55] |
| | Mutation of PBP1 penicillin binding domain | Ala369Thr | [63] |
| | | Val374Leu | [63] |
| | | Glu406Ala | [59] |
| | | Ser414Arg | [58, 63] |
| | | Ser417Thr | [59] |
| | | Leu423Phe | [63] |
| | | Thr438Met | [60] |
| | | Met515Ile | [59] |
| | | Asp535Asn | [59] |
| | | Ser543Arg | [59] |
| | | Thr556Ser | [59, 63] |
| | | Asn562Tyr | [59, 63] |
| | | Thr593Ala | [63] |
| | | Gly595Ser | [63] |
| | | Lys648Gln | [59] |
| | | Arg649Lys | [59] |
| | | Arg656Pro | [59] |
| | Thr438Met | [60] | |
| Mutation of PBP1 and PBP3 | PBP1: Ser414Arg, Leu423Phe, Asn562Tyr, and Thr593Ala PBP3: Ala499Val and Glu536Lys | [65] | |
| Mutation of PBP1, PBP2, and PBP3 | PBP1: Ser414Arg, Leu423Phe, Asn562Tyr, and Thr593Ala PBP2: Ala296Val, Asn/Ser494His, Ala/Val541Met, | [65] | |

| Antibiotic Class | Mechanism of resistance | Mutation | Reference |
|--|---|---|--------------------------|
| | | and Glu572Gly PBP3: Ala499Val and Glu536Lys | |
| | Decrease membrane permeability | Mutations in Amino Acids 116–201 of <i>hopB</i> | [60] |
| | | Stop codon at amino acid 211 of <i>hopC</i> | [60] |
| Nitroimidazoles | Inactivation of <i>rdxA</i> | Various mutations producing truncated RdxA | [73, 75–82, 177] |
| | Mutation of <i>rdxA</i> | Arg16His | [80] |
| | | Gln197Lys | [80] |
| | | Cys19Tyr | [77] |
| | | Tyr46His | [79] |
| | | Pro51Leu | [79] |
| | | Ala67Val | [79] |
| | | Ala80Thr | [80] |
| | Double mutation of Ala80Thr and Val204Ile | [80] | |
| | Inactivation of <i>frxA</i> | Various mutations producing truncated FrxA | [84, 85] |
| | Missense mutations in <i>frxA</i> | Cys161Tyr | [85] |
| | | Arg206His | [85] |
| | | Double mutation of Trp137Arg and Glu164Gly | [85] |
| Dual inactivation of <i>frxA</i> and <i>rdxA</i> | Various mutations producing truncated RdxA and FrxA | [77, 84, 85] | |
| | Unknown | [83] | |
| Dual inactivation of <i>fdxB</i> and <i>rdxA</i> | Unknown | [83] | |
| Tetracyclines | Mutation of primary tetracycline binding site in 16S rRNA gene | Mutations of Nucleotides AGA926–928 TTC | [103–107] |
| | | Various single and double mutations of nucleotides 926– 928 | [99, 105, 106, 108, 109] |
| | Unknown | Unknown | [108, 109] |
| Fluoroquinolones | Mutations in <i>gyrA</i> | Asn87Lys | [116–118] |
| | | Asn87Ile | [120] |
| | | Asn87Tyr | [120] |
| | | Ala88Val | [116–118] |
| | | Asp91Ala | [117] |
| | | Asp91Asn | [116–118] |
| | | Asp91Gly | [116–118] |
| | | Asp91Tyr | [116–118] |
| | | Double mutation of Ser83Ala and Asn87Lys | [118]. |
| | | Double mutation of Ala84Pro and Ala88Val | [118]. |

| Antibiotic Class | Mechanism of resistance | Mutation | Reference |
|------------------|-----------------------------|---|------------|
| | | Double mutation of Asn87His and Asp91Gly | [120] |
| | | Double mutation of amino acid Asp91Gly and Ala97Val or Ala97Asn** | [116, 118] |
| | | Double mutation of amino acid Asp91Asn and Ala97Val or Ala97Asn** | [116, 118] |
| | | Double mutation of amino acid Asp91Tyr and Ala97Val or Ala97Asn** | [116, 118] |
| | Unknown | Unknown | [118] |
| Rifamycins | Mutations in <i>rpoB</i> | Val149Phe | [128, 129] |
| | | Leu525Pro | [126] |
| | | Gln527Arg | [126, 127] |
| | | Gln527Lys | [126] |
| | | Asp530Asn | [126] |
| | | Asp530Val | [126, 127] |
| | | His540Asn | [126] |
| | | His540Tyr | [126, 127] |
| | | Ser545Leu | [126, 127] |
| | | Ile586Asn | [126] |
| | | Ile586Leu | [126] |
| Arg701His | [129] | | |
| Nitrofurans | Mutation in nitroreductases | Mutations in PorCDAB and OorDABC | [133] |

* Indicates that there is controversy over the role of this mutation [40].

** Indicates that a single mutation at amino acid 97 was not sufficient to confer resistance [119].