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Review Paper

Molecular Mechanisms of Antibiotic Resistance in *Helicobacter pylori*

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Abstract

Helicobacter pylori are a Gram-negative rod which colonizes the stomach of approximately half the world's population. First identified in 1983 as a pathogen, it has now been accepted as the causative agent of several gastric disorders ranging from chronic active gastritis and peptic ulcer disease to gastric cancer. The recognition of as a pathogen has had a significant impact on gastroenterologic practice and has made diagnosis and treatment of clinically relevant. Although H. pylori are susceptible to many antibiotics in vitro, only a few antibiotics can be used in vivo to cure infected patients. The frequent indication for eradication therapy and the limited choice of antibiotics have resulted in the development of antibiotic resistance in H. pylori, which significantly impairs the treatment of H. pylori-associated disorders. The prevalence of antibiotic resistance of shows regional variation per antibiotic, but can be as high as 95%. In this review the clinical implications, the molecular mechanisms, and recently developed molecular detection methods for antibiotic resistance in are discussed.

Keywords: Helicobacter pylori, antibiotic resistance, prevalence, gastritis and PCR.

Introduction

In 1983, Warren and Marshall were the first to report the successful cultivation of the human pathogen *Helicobacter pylori* from gastric biopsy samples. They went on to fulfill Koch's postulates in a self-infection experiment, in which they demonstrated that colonization results in active gastritis, which could be cured by treatment with antibiotics (Marshall*et al*., 1985). This important discovery has significantly changed the management of gastroduodenal diseases and has in particular changed peptic ulcer disease from a chronic, relapsing disease of

uncertain cause to a curable infectious condition. Infection with occurs worldwide. In industrialized countries 20% to 50% of the middle-aged adults are infected with *H. pylori*, compared to 80% or more in manydeveloping countries. In the latter, most people become colonized with before the age of ten, whereas in industrialized countries only a few children are infected at this age (Rothenbacher and Brenner *et al.*, 2003). In industrialized countries, the incidence of infections has decreased substantially over the recent decades, probably due to improved socio-economic status, sanitation and/or living conditions. Therefore, the increase in prevalence of with age seen in the industrialized countries is generally explained by a cohort effect. Infection with is associated with an acute pangastritis. This acute gastritismay be symptomatic with nausea, vomiting and abdominal pain. Although the infection is cleared in some patients, it progresses to chronic active gastritis in most subjects. In a proportion of them, this eventually gives rise to peptic ulcer disease, and atrophic gastritis, and gastric adenocarcinoma (Kuipers *et al.*, 1995). Furthermore, infections have been associated with gastric B-cell mucosa associated lymphoid tissue (MALT) lymphoma, hypertrophicgastropathy, and hypochlorhydria.

Treatment of Infections

Peptic ulcer disease and other *H. pylori*-associated disorders usually regress or heal completely after eradication of with antibiotics. In vitro, *H. pylori* are susceptible to the majority of antibiotics but in vivo most antibiotics are unable tocure infected patients. This is thought to be due to a combination of factors, including (i) the inability of drugs to achieve appropriate levels in the gastric mucus layer, (ii) inactivation of drugs at low pH and (iii) the slow growth rate of *H. pylori* (Gisbert and Pajares, 2003).

Successful eradication of therefore requires combination therapy, consisting of one or more antibiotics in combination with an acid-suppressive drug and/or a bismuth component. Inclusion of an acid-suppressive drug was shown to increase the efficacy of the combination therapy. There is a preference for the use of proton pump inhibitors (PPI), such as omeprazole and lansoprazole rather than H_2-receptor antagonists, eventhough H_2-receptor antagonists-based regimens, in particular those with ranitidine, were shown to be equally effective as PPI-based ones. Acid-suppressive drugs primarily increase the intragastric pH, and enhance the activity of the antibiotics. An additional beneficial effect of acid-suppressive drugs is that they decrease the severity of side effects of a given regimen (Borody *et al.*, 1995), resulting in an increased compliance and chance for successful treatment.

Bismuth salts have already been used in medicine since the 19th century, particularly in the treatment of peptic diseases. Colloidal bismuth substrate, bismuth subsalicylate andthe newer ranitidine bismuth citrate (acid inhibitor combined with a bismuth compound) are commonly used agents in anti-therapy. The mode of action of bismuth salton is complex and includes inhibition of protein, ATP, and cell wall synthesis. Although bismuth monotherapy effectively suppresses growth of H. pylori, the eradication rates with this therapy are low (Gorbach *et al.*, 1990). However, when used in combination with one or two antibiotics, these compounds have synergistic activity. While bismuth-based triple and quadruple therapies seem to be even more effective against infection than PPI-based

therapies, they are usually not given as first-line treatment, because of their more complex dosing schedule and/or frequent side effects (Gisbert and Pajares, 2001).

Triple therapies consisting of two of the previous mentioned antibiotics, and a PPI or ranitidine bismuth citrate for 7-10 days are now mostly recommended. There seems to be preference for combination therapy that includes amoxicillin and clarithromycin. Despite good results in many clinical trails, these therapies are not always as successful in daily clinical practice. Failure of first-line therapy is usually related to insufficient patient compliance and/or development of antibiotic resistance. For retreatment, 10 to 14 day treatment courses with higher doses of the antibiotics and/or bismuth component is often advised, usually with inclusion of previously unused antibiotics (Gisbertand Pajares, 2003).

Most patients who remain H. pylori-positive after two consecutive courses of eradication treatment have been infected with a strain that is resistant to one or more of the previously used antibiotics (Megraud and Doermann, 1998;Van Der Wouden *et al.*, 2000). To select an appropriate third-line treatment, endoscopy followed by bacterial culture and antibiotic susceptibility testing is advisable.

Prevalence of Antimicrobial Resistance

As antibiotic resistance in seems to be the main reason of therapy failure, detection of antibiotic resistance is of importance. Antibiotic susceptibility of is usually assessed by culture-based methods such as E-test, agar dilution and disc diffusion. These methods offer the opportunity to determine the minimal inhibitory concentration (MIC) of antibiotics, but on the other hand they are time-consuming and results are not always consistent (Menard, *et al*., 2002). Factors such as cell viability, inoculation size, incubation conditions, and growth media may affect their outcome (Hao*et al*., 2004). Molecular-based methodsare independent of these factors, and thus they offer an attractive alternative. These testsgive reproducible results and are easily standardized. Moreover, they are faster than the conventional culture-based assays, and when applied directly on gastric biopsy samples, data can be obtained at the day of endoscopy. However, most studies originated from single centers, included only a small number of strains, were often restricted to selected patients, and used different techniques to assessantibiotic susceptibility. Antibiotic resistance rates should preferentially be obtained fromlarge-scale multicenter surveillance programs using standardized detection methods in order to reduce the probability of under- or overestimation of the prevalence of antibiotic resistance in *H. pylori*. However, these surveillance programs are expensive; and only performed in few countries (Wolle*et al*., 2002) more often investigator driven rather than government induced. Antibiotic resistance in is already widespread and increasing. Metronidazoleresistance (MIC > 8 mg/L) is the most common antimicrobial resistance in H. pylori. Inindustrialized countries approximately 35% of the strains are metronidazoleresistant, whereas in developing countries, the resistance rates formetronidazole are very high, and in some areas virtually all strains are metronidazole-resistant (Frenck and Clemens, 2003). In comparison with metronidazole resistance, the prevalence of clarithromycin resistance (MIC $\geq$ 2 mg/L) in is much lower. In industrialized countries, approximately 10% of the strains are clarithromycin-resistant (Frenck and Clemens, 2003).

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Molecular Mechanisms of Antibiotic Resistance

Metronidazole Activity and Mechanism of Resistance

Metronidazole was initially promoted for the treatment of gynaecological infectionscaused by Trichomonasvaginalis, but soon it became apparent that the drug was alsoactive against anaerobic and some microaerophilic bacteria (Frenck and Clemens, 2003).As metronidazole isactively released into the gastric juice and its antimicrobial activity is only marginally affected by low pH (Debets-Ossenkopp, 1999), this drug is highly effective against *H. pylori*. This reduction leads to the formation of nitro-anion radicals and metronidazole intermediates that cause lethal damage to sub cellular structures and DNA. Theoretically, any protein that possesses a low redox potential can accept electrons from metronidazole, and thus activate the drug. In several putative electron acceptors have been identified, including ferredoxin(fdxA), ferredoxin-like protein (fdxB), flavodoxin (fldA), NAD(P)H flavinnitroreductase(frxA), 2-oxoglutarate oxidoreductase (oorD), pyruvate:ferredoxinoxidoreductase (porD), and oxygen-insensitive NAD(P)H nitroreductase (rdxA) (Alm*et al*., 1999). In *H. pylori*, levels of metronidazole resistance are very diverse, with MICs ranging from 8 to ≥ 256 mg/L. This spread in MIC values suggests that high-level metronidazole resistance can result from mutational changes in one locus, but it is more likely that several pathways are involved. Potential mechanisms of metronidazole resistance studied in include (i) deficient drug uptake and/or increased drug efflux ; (ii) enhanced activity of DNA repair enzymes;(iii) increased oxygen scavenging capabilities ; and (iv) decreased drug activation arising from changes in metronidazole-reducing enzymes (Goodwin *et al.*,1998;Kwon *et al*, 2000).

Metronidazole is a typical substrate of resistance-nodulation-division (RDN) multipledrug efflux mechanism that plays a role in the antibiotic resistance of several bacteria. In the genome three putative RDN efflux systems have been identified. When these three RDN operons were mutated, it did not affect the in vitro susceptibility of to nineteen antibiotics. Although metronidazole was not included in this study, it was suggested that the expression levels of these proteins were too low to influence antibiotic susceptibility in general (Bina*et al*., 2000).

The recA gene encodes a protein that plays an essential role in DNA recombination and DNA repair. In *H. pylori*, a recA homologue has been identified and functionally characterized. Mutants with an inactivated recA gene are severely impaired in their ability to survive UV light treatment and exhibit enhanced susceptibility to metronidazole (Thompson *et al.*, 1995). Although transfer of the recA gene of several metronidazole-resistant strains to metronidazole-susceptible strains resulted in an increased MIC of metronidazole, sequence analysis did not established that observed resistance was caused by mutational changes in the recA gene(Chang *et al*.,1997).An important step in the elucidation of the molecular mechanism of metronidazoleresistance in came with the discovery that null mutations in the rdxAgene induced metronidazole resistance in a formerly metronidazole-susceptible strain. Many studies have subsequently shown that the majority of metronidazole-resistant strains contain various mutations within the rdxA gene, including frameshifts, premature stop codons, insertion of transposable elements,

codon changes resulting in amino acid substitution, and promoter alterations (Bereswill*et al*., 2003; Debets*et al*., 1998). As metronidazole-resistant isolates in vitro become metronidazole susceptible after exposure to short periods of anaerobiosis, it was suggested thatmetronidazole susceptibility may be restored at low oxygen conditions through the activation of potential anaerobic reduction pathways that function less, or not all, undermicroaerophilic conditions. (Smith and Edwards, 1995).

Clarithromycin activity and mechanism of resistance

Clarithromycin is a bacteriostatic antibiotic that belongs to a group of macrolides that bind to peptidyltransferase loop of domain V the 23S rRNA molecule. This binding interferes with protein elongation, and thus effectively blocks bacterial protein synthesis. The antibacterial activity of clarithromycin is similar to that of other macrolides, but clarithromycin is better absorbed in the gastric mucus layer, more acid-stable, and therefore more effective against (Paul *et al.*, 2001).Resistance to clarithromycin in is caused by point mutations in two adjacent 23SrRNA nucleotides, namely 2142 and 2143. It can be induced by an adenine (A) to guanine (G) substitution at one of these positions or an adenine (A) to cytosine (C) substitution solely at position 2142. In these substitutions cause decreased affinity of the ribosomes for several macrolides, resulting in increased resistance (Occhialini*et al*., 1997). The A2142G and A2142C (underlined letters are used to represent the basepair changes) were significantly more present in isolates with a higher MIC ofclarithromycin (> 64 mg/L), whereas the A2143G substitution was often found in isolates with a lower MIC (< 64 mg/L). Occasionally, other 23S rRNAmutations have also been reported for H. pylori; some of them are associated with high level resistance, while others are associated with low-level resistance. To define the apparently high prevalence of the A to G at position 2142 and 2143 among clarithromycin-resistant clinical isolates, site-directed mutants were created containing either a G, C, or T (thymine) substitution at position 2142 or 2143. In a similar isogenic background it was seen that the preference for A to G substitutions results from higher growth rates, higher MICs and more stable resistance (Debets*etla*., 1998;Wolle*et al*., 2002).

Heterogeneity still results in clarithromycin resistance, but it generally appears to be associated with lowerresistance levels than present in homogenicisolates. The higher prevalence of homogeneity over heterogeneity in may reflect a high efficiency of DNA recombination in this organism. The mutation in one copy of the 23S rRNA may be easily transferred to the other 23S rRNA gene by efficient homologous DNA recombination under selective pressure, conferring higher levels of clarithromycin resistance.As expected, clarithromycin resistance coincides with resistance to other macrolides. The A2142G and A2142C mutations are linked to high-level cross-resistance to all macrolides, whereas the A2143G mutation give rise to high-level resistance to erythromycin and intermediate-level resistance to clindamycin and streptogramin (Garcia *et al*., 1999; Wang and Taylor *et al.*, 1998).

Amoxicillin activity and mechanism of resistance

Amoxicillin is a bactericidal antibiotic that belongs to group of penicillins. It binds to penicillin-binding proteins (PBPs), and thus interferes with bacterial cell wall synthesis,resulting in the lyses of replicating bacteria. The antibacterial affect of amoxicillin issimilar to that of other β-lactam antibiotics, but amoxicillin is released in the gastric juice and thus frequently used in anti-therapy. Until recently, it was thought that amoxicillin resistance in did

not exist, although resistance to thisantimicrobial drug could be induced by continuous exposure of to amoxicillin in vitro (Chang *et al*., 2001;Paul *et al*., 2001). PBPs are enzymes that are involved in the synthesis and maintenance of thepeptidoglycan layer of the bacterial cell wall (Hao*et al*., 2004). In nine putative PBPs havebeen identified; three high-molecular-weight PBPs and six low-molecular-weight PBPs. Two of these proteins have been associated with amoxicillin resistance in *H. pylori*. At first, the molecular mechanism was described for amoxicillin-resistant isolates (MIC > 256 mg/L) obtained from dyspeptic patients from Italy and the United States. As these isolates lost their resistance phenotype upon freezing at -80°C (Dore *et al*., 1999), they are often referred to amoxicillin-tolerant. In addition to changes in the pbp1A gene, it was postulated that reduced membranepermeability and/or active efflux of amoxicillin in might also contribute to higher levels of amoxicillin resistance. Both aspects have been tested in a quantitativeuptake experiment using the proton translocator CCCP. In this experiment it was seen that amoxicillin-resistant strains accumulated <60% penicillin G compared toamoxicillin-susceptible strains, both in the presence and absence of CCCP; excluding the role of an active efflux mechanism. This suggests that amoxicillin resistance in part is due to an increased diffusional barrier an effect that may be explained by alterations in outer membrane proteins (Kwon *et al* ., 2003).

Tetracycline activity and mechanism of resistance

Tetracycline is a bacteriostatic antibiotic that is active against several Gram-positive and Gram-negative bacteria, including H. pylori. It accumulates in the cytoplasm, where it binds to the 30S ribosomal subunit. Here it interferes with the attachment ofaminoacyl-tRNA to the ribosome, and inhibits protein synthesis and bacterial growth. Tetracycline is an antibiotic that is extensively used in many industrialized countries, and as a consequence resistance to this drug has become an emerging problem. In general, four tetracycline resistance mechanisms have been identified: (i) deficient drug uptake and/or increased drug efflux; decreased antibiotic binding by (ii) changes in ribosomal protection proteins, or (iii) mutations 16S rRNA tetracycline binding site; and (iv) enzymatic inactivation of tetracycline. In many bacteria, resistance to tetracycline is due to an energy-dependent efflux oftetracycline-cation complexes across the cell membrane by membrane-associated proteins. The exchange of tetracycline-cations by protons reduces the intracellular drug concentration and protects ribosomes against tetracycline. Overexpression of the efflux proteins often confers multidrug resistance, while deletions in these operons increases tetracycline susceptibility (Chopra and Roberts, 2001). The second common mechanism that mediates tetracycline resistance acts by ribosomal protection proteins. These cytoplasmic proteins confer tetracycline resistance either by reduced affinity of ribosomes for tetracycline, or by released binding of the drug to the ribosomes.

Activity of to Less Commonly Used Antibiotics and their Mechanism of Resistance

As resistance against the four commonly used antibiotics of the anti-therapy is increasing, fluoroquinolones, nitrofurans and rifamycins are occasionally being introduced in second- and third-line therapies. Initial results obtained with these antibiotics were promising, but soon the eradication rates dropped as resistance against these drugs developed (Debets*et al*., 1999;Jeong*et al*., 2001).

Fluoroquinolones

Fluoroquinolones (i.e. ciprofloxacin, moxifloxacin, trovafloxacin and levofloxacin) are bactericidal antibiotics that exert their antimicrobial activity by inhibiting the enzyme DNAgyrase. This enzyme is a tetramer consisting of twoA subunits and two B subunits, encoded by the gyrAand gyrB genes, respectively. The main function of this enzyme is to catalyze the negative super coiling of DNA. Most isolates are susceptible to fluoroquilones, but the incidence of fluoroquinolone resistance seems to increase (Nakamura *et al*., 2003; Tindberg*et al* ., 2001).

Nitrofurans

Furazolidone and nitrofurantoin are nitroheterocyclic and nitroaromatic compounds that share similarities with metronidazole both in their structures and modes of action is usually susceptible to furazolidone and nitrofurantoin, but occasionally strainswith an increased MIC have been reported . The resistance mechanism of furazolidone and nitrofurantoin is currently unknown, but it is clear that it differs from that of metronidazole, since inactivation of the rdxA, frxA and fdxBgenes did not result in furazolidone or nitrofurantoin resistance (Kwon *et al* .,2001).

Rifamycins

Rifabutin and several other derivates of rifampin are bactericidal antibiotics that bind tothe β-subunit of DNA-dependent RNA polymerase, resulting in inhibition of transcription. The β-subunit of this complex is encoded by the rpoB gene. Until a few years back resistance against rifamycins and rifabutin in vivo was very rare, however, the incidence of rifamycin and rifabutin resistance is increasing. In resistance to these antibiotics is linked to various point mutations in the rpoB gene (corresponding to amino acid codon 149, 524-545 and 586) (Heep*et al*., 2000).

Molecular Detection of Antibiotic Resistance

Since in most resistances is due to specific mutations, molecular-based methods offer an attractive alternative to the conventional culture-based methods. Molecular-basedmethods are independent of cell viability or growth rate of the bacteria, and thus more consistent and reproducible than the culture-based ones. Moreover, molecular-basedmethods are much faster, and if directly applied to gastric biopsy specimens, results canbe obtained at the day of endoscopy. Although DNA sequencing is the gold standard for the detection of mutational changes, this method is not cost effective in a routine setting.The development of rapid, genotype-based tests for metronidazole resistance in is not easy, because the resistance is associated with various unrelated mutations within the rdxA gene and other reductase-encoding genes. Despite these barriers, there is newevidence that indicates that a system could be developed based on detection of the RdxAprotein. Using immunoblot with specific anti-RdxA antibody a 24 kDaimmunoreactive band was observed in all metronidazole-susceptible isolates but was absent in most (90%) metronidazole-resistant isolates (Latham *et al* ., 2002).

Most assays are polymerase chain reaction (PCR)-based using different methods to study the amplicons. Restriction fragment length polymorphism (RFLP) is asimple method that is based on the occurrence of restriction site within the amplicon. This assay allows for the detection of the previously mentioned 23S rRNA mutations using the restriction endonucleases, MboII (A2142G), BbsI (A2142G), BsaI (A2143G) and BceAI(A2142C) (Menard *et a*l .,

2002;Mendonca*et al*.,2000;Mendz and Megraud, 2002).Other methods, such as PCR-DNA enzyme immunoassay (DEIA), PCR oligonucleotideligation assay (OLA), preferential homoduplex formation (PHFA) and PCR-line probeassay (LipA), include an additional hybridization step after the PCR. The PCR productswere hybridized with labeled oligonucleotide probes under highly stringent conditions and hybrids were subsequently detected with specific antibodies or streptavidin-alkaline phosphatase. Recently, several real-time PCR hybridization assays have been developed. In these assays a 23S rDNA fragment is amplified in the presence of a fluorescent-labeled mutation and anchor probe. When these probes hybridize with the PCR product, a fluorescence signal is emitted. After completion of the PCR, the temperature is increased to determine the melting point of the mutation probe. The temperature at which the fluorescent signal drops indicates the point at which the mutation probe dissociates (melting point). When there are mismatches present in the target sequence, lower melting temperatures are obtained compared to the matched hybrid. This technique is simple and quick, and if applied directly on gastric tissue, results can be obtained within 3 hours (Mendonca*et al*., 2000). There is also a possibility to detect clarithromycin resistance without performing PCR, by using fluorescence in situ hybridization (FISH). In this assay intact are hybridized with fluorescent-labeled H. pylori-specific 16S and 23S rRNA probes. Thelabeled bacteria were subsequently visualized by fluorescence microscopy. This assayallows detection of and clarithromycin resistance simultaneously.

Conclusions

Antibiotic resistance in is widespread and further increasing. This constitutes a considerable clinical problem, as antibiotic resistance negatively affects the efficacy of anti-therapy. For this reason antibiotic resistance in shouldbe monitored. Data obtained in such a surveillance program are of great value to guide anti-therapy and help to gain a better understanding of the effect of resistance on therapy outcome. However, in most countries, including The Netherlands, these surveillance programs are lacking, and therapy recommendations may be based on insufficient data. Conventional culture-based susceptibility testing of is relatively slow and cumbersome. Thus molecular-based methods offer an attractive alternative.

It is likely that real-time PCR will have an expanding role in the rapid detection of antibiotic resistance in H. pylori, particularly if results are obtained directly from gastric biopsy samples without the need for cultivation. Since for amoxicillin and metronidazole resistance in no appropriate tests are available yet, research on the elucidation of molecular mechanisms and development of molecular detection methods should be continued.

As novel antibiotics are not rapidly forthcoming the search for new antimicrobial agents and co-therapies should be intensified. Antimicrobial peptides porphyries essential oils but also probiotics may prove to be helpful.

References

[1]Alm, R. A., L. S. Ling, D. T. Moir, B. L. King, E. D. Brown, P. C. Doig, D. R. Smith, B. Noonan, B. C. Guild, B. L. deJonge, G. Carmel, P. J. Tummino, A. Caruso, M. UriaNickelsen, D. M. Mills, C. Ives, R. Gibson, D. Merberg, S. D. Mills, Q. Jiang, D. E. Taylor, G. F. Vovis, and T. J. Trust. 1999. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen Helicobacter pylori. Nature 397:176-180.

[2]Bereswill, S., C. Krainick, F. Stahler, L. Herrmann, and M. Kist. 2003. Analysis of the rdxA gene in high-level metronidazole-resistant clinical isolates confirms a limited use of rdxA mutations as a

marker for prediction of metronidazole resistance in Helicobacter pylori. FEMS Immunol. Med. Microbiol. 36:193-198.

[3]Bina, J. E., R. A. Alm, M. Uria-Nickelsen, S. R. Thomas, T. J. Trust, and R. E. Hancock. 2000. Helicobacter pylori uptake and efflux: basis for intrinsic susceptibility to antibiotics in vitro. Antimicrob.Agents Chemother. 44:248-254.

[4]Borody, T. J., P. Andrews, G. Fracchia, S. Brandl, N. P. Shortis, and H. Bae. 1995. Omeprazole enhances efficacy of triple therapy in eradicating Helicobacter pylori. Gut 37:477-81.

[5]Chang, K. C., S. W. Ho, J. C. Yang, and J. T. Wang. 1997. Isolation of a genetic locus associated with metronidazole resistance in Helicobacter pylori. Biochem.Biophys.Res.Commun. 236:785-8.

[6]Chopra, I., and M. Roberts. 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol.Mol. Biol. Rev. 65:232-260.

[7]Debets-Ossenkopp, Y. J., A. B. Brinkman, E. J. Kuipers, C. M. Vandenbroucke-Grauls, and J. G. Kusters. 1998. Explaining the bias in the 23S rRNA gene mutations associated with clarithromycin resistance in clinical isolates of Helicobacter pylori. Antimicrob.Agents Chemother. 42:2749-2751.

[8]Debets-Ossenkopp, Y. J., F. Namavar, and D. M. MacLaren. 1995. Effect of an acidic environment on the susceptibility of Helicobacter pylori to trospectomycin and other antimicrobial agents. Eur. J. Clin. Microbiol. Infect. Dis. 14:353-355.

[9]Debets-Ossenkopp, Y. J., R. G. Pot, D. J. van Westerloo, A. Goodwin, C. M. VandenbrouckeGrauls, D. E. Berg, P. S. Hoffman, and J. G. Kusters. 1999. Insertion of mini-IS605 and deletion of adjacent sequences in the nitroreductase (rdxA) gene cause metronidazole resistance in Helicobacter pylori NCTC11637. Antimicrob.Agents Chemother. 43:2657-2662.

[10]Debets-Ossenkopp, Y. J., R. G. Pot, D. J. van Westerloo, A. Goodwin, C. M. VandenbrouckeGrauls, D. E. Berg, P. S. Hoffman, and J. G. Kusters. 1999. Insertion of mini-IS605 and deletion of adjacent sequences in the nitroreductase (rdxA) gene cause metronidazole resistance in Helicobacter pylori NCTC11637. Antimicrob.Agents Chemother.43:2657- 2662.

[11]Dore, M. P., M. S. Osato, G. Realdi, I. Mura, D. Y. Graham, and A. R. Sepulveda. 1999. Amoxycillin tolerance in Helicobacter pylori. J. Antimicrob. Chemother. 43:47-54.

[12]Frenck, R. W., Jr., and J. Clemens. 2003. Helicobacter in the developing world. Microbes Infect. 5:705-13. Fallone, C. A. 2000. Epidemiology of the antibiotic resistance of Helicobacter pylori in Canada. Can. J. Gastroenterol. 14:879-82.

[13]Garcia-Arata, M. I., F. Baquero, L. de Rafael, C. Martin de Argila, J. P. Gisbert, F. Bermejo, D. Boixeda, and R. Canton. 1999. Mutations in 23S rRNA in Helicobacter pylori conferring resistance to erythromycin do not always confer resistance to clarithromycin. Antimicrob.Agents Chemother. 43:374-6.

[14]Gisbert, J. P., and J. M. Pajares. 2001. Helicobacter pylori therapy: first-line options and rescue regimen. Dig. Dis. 19:134-143.

[15]Gisbert, J. P., and J. M. Pajares. 2003. Treatment of Helicobacter pylori eradication failures. Curr. Treat. Options Gastroenterol. 6:147-156

[16]Goodwin, A., D. Kersulyte, G. Sisson, S. J. Veldhuyzen van Zanten, D. E. Berg, and P. S. Hoffman. 1998. Metronidazole resistance in Helicobacter pylori is due to null mutations in a gene (rdxA) that encodes an oxygen-insensitive NADPH nitroreductase. Mol. Microbiol. 28:383-393.

[17]Gorbach, S. L. 1990. Bismuth therapy in gastrointestinal diseases. Gastroenterology 99:863-75

[18]Hao, Q., Y. Li, Z. J. Zhang, Y. Liu, and H. Gao. 2004. New mutation points in 23S rRNA gene associated with Helicobacter pylori resistance to clarithromycin in northeast China. World J. Gastroenterol. 10:1075-7.

[19]Heep, M., M. Kist, S. Strobel, D. Beck, and N. Lehn. 2000. Secondary resistance among 554 isolates of Helicobacter pylori after failure of therapy. Eur. J. Clin. Microbiol. Infect. Dis. 19:538-41.

[20]Jeong, J. Y., A. K. Mukhopadhyay, J. K. Akada, D. Dailidiene, P. S. Hoffman, and D. E. Berg. 2001. Roles of FrxA and RdxAnitroreductases of Helicobacter pylori in susceptibility and resistance to metronidazole. J. Bacteriol. 183:5155-5162.

[21]Kuipers, E. J., A. M. Uyterlinde, A. S. Pena, R. Roosendaal, G. Pals, G. F. Nelis, H. P. Festen,and S. G. Meuwissen. 1995. Long-term sequelae of Helicobacter pylori gastritis. Lancet 345:1525-8.

[22]Kwon, D. H., F. A. El Zaatari, M. Kato, M. S. Osato, R. Reddy, Y. Yamaoka, and D. Y. Graham. 2000. Analysis of rdxA and involvement of additional genes encoding NAD(P)H flavinoxidoreductase (FrxA) and ferredoxin-like protein (FdxB) in metronidazole resistance of Helicobacter pylori. Antimicrob.Agents Chemother. 44:2133-2142.

[23]Kwon, D. H., M. Lee, J. J. Kim, J. G. Kim, F. A. El Zaatari, M. S. Osato, and D. Y. Graham. 2001. Furazolidone- and nitrofurantoin-resistant Helicobacter pylori: prevalence and role of genes involved in metronidazole resistance. Antimicrob.Agents Chemother. 45:306-308.

[24]Kwon, D. H., M. P. Dore, J. J. Kim, M. Kato, M. Lee, J. Y. Wu, and D. Y. Graham. 2003. High-level beta-lactam resistance associated with acquired multidrug resistance in Helicobacter pylori. Antimicrob.Agents Chemother. 47:2169-78.

[25]Latham, S. R., A. Labigne, and P. J. Jenks. 2002. Production of the RdxA protein in metronidazole-susceptible and - resistant isolates of Helicobacter pylori cultured from treated mice. J. Antimicrob. Chemother. 49:675-678.

[26]Marshall, B. J., J. A. Armstrong, D. B. McGechie, and R. J. Glancy. 1985. Attempt to fulfil Koch's postulates for pyloric Campylobacter. Med. J. Aust. 142:436-9.

[27]Megraud, F., and H. P. Doermann. 1998. Clinical relevance of resistant strains of Helicobacter pylori: a review of current data. Gut 43 Suppl 1:S61-S65.

[28]Menard, A., A. Santos, F. Megraud, and M. Oleastro. 2002. PCR-restriction fragment length polymorphism can also detect point mutation A2142C in the 23S rRNA gene, associated with Helicobacter pylori resistance to clarithromycin. Antimicrob.Agents Chemother. 46:1156-7.

[29]Mendonca, S., C. Ecclissato, M. S. Sartori, A. P. Godoy, R. A. Guerzoni, M. Degger, and J.Pedrazzoli, Jr. 2000. Prevalence of Helicobacter pylori resistance to metronidazole, clarithromycin, amoxicillin, tetracycline, and furazolidone in Brazil. Helicobacter 5:79-83.

[30]Mendz, G. L., and F. Megraud. 2002. Is the molecular basis of metronidazole resistance in microaerophilic organisms understood? Trends Microbiol. 10:370-375.

[31]Nakamura, M., R. C. Spiller, D. A. Barrett, J. I. Wibawa, N. Kumagai, K. Tsuchimoto, and T. Tanaka. 2003. Gastric juice, gastric tissue and blood antibiotic concentrations following omeprazole, amoxicillin and clarithromycin triple therapy. Helicobacter 8:294-9.

[32]Occhialini, A., M. Urdaci, F. Doucet-Populaire, C. M. Bebear, H. Lamouliatte, and F.Megraud. 1997. Macrolide resistance in Helicobacter pylori: rapid detection of point mutations and assays of macrolide binding to ribosomes. Antimicrob.Agents Chemother. 41:2724-2728.

[33]Paul, R., S. Postius, K. Melchers, and K. P. Schafer. 2001. Mutations of the Helicobacter pylori genes rdxA and pbp1 cause resistance against metronidazole and amoxicillin. Antimicrob.Agents Chemother. 45:962-965.

[34]Rothenbacher, D., and H. Brenner. 2003. Burden of Helicobacter pylori and H. pylori-related diseases in developed countries: recent developments and future implications. Microbes Infect. 5:693-703.

[35]Smith, M. A., and D. I. Edwards. 1995. The influence of microaerophilia and anaerobiosis on metronidazole uptake in Helicobacter pylori. J. Antimicrob. Chemother. 36:453-61.

[36]Thompson, S. A., and M. J. Blaser. 1995. Isolation of the Helicobacter pylori recA gene and involvement of the recA region in resistance to low pH. Infect. Immun. 63:2185-93.

[37]Tindberg, Y., C. Bengtsson, F. Granath, M. Blennow, O. Nyren, and M. Granstrom. 2001. Helicobacter pylori infection in Swedish school children: lack of evidence of child-to-child transmission outside the family. Gastroenterology 121:310-6.

[38]Van Der Wouden, E. J., J. C. Thijs, A. A. Van Zwet, and J. H. Kleibeuker. 2000. Review article: nitroimidazole resistance in Helicobacter pylori. Aliment.Pharmacol.Ther. 14:7-14.

[39]Wang, G., and D. E. Taylor. 1998. Site-specific mutations in the 23S rRNA gene of Helicobacter pylori confer two types of resistance to macrolide-lincosamide-streptograminB antibiotics. Antimicrob.Agents Chemother. 42:1952-8.

[40]Wolle, K., A. Leodolter, P. Malfertheiner, and W. Konig. 2002. Antibiotic susceptibility of Helicobacter pylori in Germany: stable primary resistance from 1995 to 2000. J. Med. Microbiol. 51:705-9.