Helicobacter pylori resistance to antibiotics: Prevalence, mechanism, detection. What's new?

Francis Mégraud MD

F Mégraud. *Helicobacter pylori* resistance to antibiotics: Prevalence, mechanism, detection. What's new? Can J Gastroenterol 2003;17(Suppl B):49B-52B.

Helicobacter pylori resistance to antibiotics is increasingly reported and may limit the efficacy of current treatment regimens. Their resistance mechanism has been found to be point mutations for all antibiotics. Macrolide resistance is the most clinically important, but can be detected efficiently by molecular methods. Metronidazole resistance has limited clinical impact but testing methods are not reliable. Seldomly found cases of resistance, such as to amoxicillin and tetracycline, have had their mechanism recently elucidated. The existance of rapid and practical methods for the detection of macrolide resistance (eg, Fluorescence Resonance Energy Transfer assay) should improve management of *H pylori*-positive patients in the future, by allowing an adapted first-line therapy.

Key Words: *Antibiotic resistance; Detection; Point mutations; Prevalence*

La prévalence, le mécanisme et la détection de l'antibiorésistance à l'*Helicobacter pylori*. Quoi de neuf ?

Il y a de plus en plus de cas d'antibiorésistance à l'*Helicobacter pylori*, et cette antibiorésistance peut limiter l'efficacité des schémas thérapeutiques. On a découvert que les mécanismes de résistance proviennent de mutations ponctuelles à tous les antibiotiques. La résistance aux macrolides est la plus importante d'un point de vue clinique, mais elle peut être décelée avec efficacité au moyen de méthodes moléculaires. La résistance au métronidazole a des répercussions cliniques limitées, mais les méthodes de test ne sont pas fiables. Le mécanisme de résistance de cas plus rares, comme ceux de résistance à l'amoxicilline ou à la tétracycline, ont été élucidés récemment. L'existence de méthodes rapides et pratiques de détection de la résistance aux macrolides (p. ex., fluorescence, essai de transfert d'énergie de résonance) devrait permettre d'améliorer la prise en charge des patients positifs au *H pylori* grâce à un traitement de première ligne adapté.

As with any infectious disease, *Helicobacter pylori* infection must be treated with antibiotics. The experience gained during the last decade has shown that a combination of a proton pump inhibitor and clarithromycin-based therapies give the best cure rates, and these are now recommended in guidelines for the treatment of this infection (1-3). The most recent guidelines, the Maastricht 2-2000 Consensus Report (4), introduced the concept of a 'treatment package', in which the second antibiotic of the first-line therapy should be amoxicillin and not metronidazole, which must be reserved in case a second-line treatment is needed.

One limitation of these treatments is compliance. In a study using Medication Event Monitoring System containers, lack of compliance occurred in more than 10% of the patients, essentially due to adverse events (5). A more important limitation is the resistance of *H pylori* to antimicrobials, especially to clarithromycin.

In the present paper, we will consider *H pylori* resistance to clarithromycin and to other antibiotics, and for each antibiotic, the global resistance rate, the impact on treatment success and new information will be presented.

RESISTANCE TO CLARITHROMYCIN

A survey performed in different countries across Europe, using the same methodology in each country (epsilometer [E]-test), showed a global clarithromycin resistance rate of 9.8%. There was a large variation between northern Europe (4.2%) and southern Europe (18.4%), with central and eastern Europe being somewhat in the middle range (9.3%) (6) (Figure 1). This can be attributed to differences in antimicrobial use. As in the case of *Streptococcus pyogenes* (7), *H pylori* resistance to macrolides seems to be dependent on the selective pressure resulting from a high consumption of these drugs (8). In Europe, a survey of children indicated a clarithromycin resistance rate of 20% (S Koletzko, personal communication).

This presents a potential problem because, when these children become adults, they will transmit strains that are already clarithromycin-resistant to their descendants and the global resistance rate may become more important.

In the United States, clarithromycin resistance rates of 12% (9) and 15% (10) have been reported, while in Canada it is 12% (11). Recent data from Asia indicate a clarithromycin resistance rate of 4%, 6% and 7.6% in Korea, Taiwan and

This article was originally presented at a conference entitled "Helicobacter pylori: Basic Mechanisms to Clinical Cure 2002", sponsored by Axcan Pharma, November 10-14, 2002, Maui, Hawaii
Laboratoire de Bactériologie, Université Victor Segalen Bordeaux 2, Bordeaux, France
Correspondence: Dr Francis Mégraud, Laboratoire de Bactériologie, Hôpital Pellegrin, Place Amélie Raba-Léon, 33076 Bordeaux cedex, France. Telephone 33-5-56-79-59-10, fax 33-5-56-79-60-18, e-mail francis.megraud@chu-bordeaux.fr

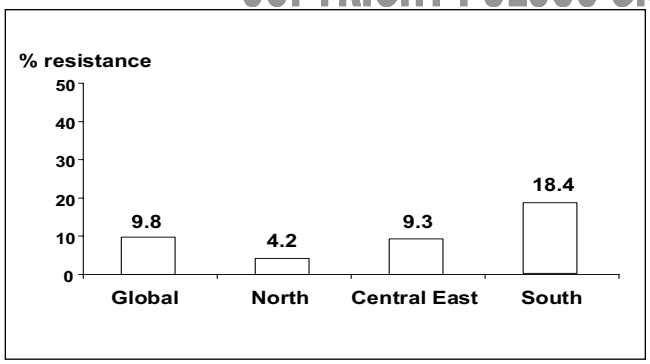

Figure 1) *Resistance of* Helicobacter pylori *to clarithromycin in Europe*

TABLE 1
Molecular methods for *Helicobacter pylori* testing of clarithromycin resistance

Based on amplification of *23S rRNA* gene
Sequencing
Restriction fragment length polymorphism (15,16)
Oligonucleotide ligation assay (17)
DNA enzyme immunoassay (18,19)
Preferential homoduplex formation assay (20)
Line probe assay (21)
Mismatch polymerase chain reaction (22)
Double gradient-denaturing gradient gel electrophoreses (23)
Fluorescence resonance energy transfer (28-30)
Based on hybridization
Fluorescence in situ hybridization assay (24)

Japan, respectively, while it was 14.4% in Australia. Macrolide resistance is important to consider because of its clinical relevance. Most of the studies comparing the cure rates in patients infected by susceptible and resistant *H pylori* strains show a huge difference, ranging from 90% success to less than 20% to 30%, respectively (10,12,13).

The mechanism of macrolide resistance is straightforward (14). Point mutations occur in two positions on the 23S rDNA (15), which change the spatial structure of the ribosome and inhibit macrolide binding (16). Mutations in other sites probably lead to nonviable bacteria or alter the fitness of the bacteria and, therefore, are not found.

In addition to the standard phenotypic methods of susceptibility testing, several molecular methods have been proposed (Table 1).

What's new?

A standard protocol to test clarithromycin susceptibility was proposed by the NCCLS several years ago (25). This protocol gives excellent results but, because agar dilution is necessary, it is difficult to implement in routine practice. For this reason, an attempt was made to validate a disk diffusion method in France. The results showed that the best method to discriminate between susceptible and resistant strains of *H pylori* is to use an erythromycin disk on a Mueller Hinton agar plate, with a critical diameter of less than 17 mm for resistant strains (26).

Polymerase chain reaction (PCR)-restriction fragment length polymorphism remains the most accessible molecular method for routine laboratories. Unfortunately, no enzyme had been described to detect the A2142C mutation. The enzyme *Bce*AI, which leads to three bands instead of two being produced when the mutation is present, was recently proposed (27).

New molecular methods have been developed. Two studies (28,29) have reported the possibility of detecting *H pylori* directly in gastric biopsies, using a real-time PCR based on the Fluorescence Resonance Energy Transfer (FRET) methodology, and of testing macrolide susceptibility by melting curve analysis (MCA) in a LightCycler apparatus (Roche Diagnostics, France) However, only a small number of biopsies with resistant strains were tested in one study, and the other study did not test for the A2142C transversion. The new FRET-MCA protocol developed in our laboratory has the capacity to detect all known mutations within two hours after receiving the biopsy, one hour being used for DNA extraction, and one hour for performing the test. We tested this protocol on 200 biopsy specimens obtained from patients who had failed eradication treatment with a clarithromycin-based triple therapy. The sensitivity and specificity compared with agar dilution were 98.4% and 94.1%, respectively (30). Interestingly, there were 42 cases with a mixture of wild type and resistant mutants, which were detected more easily than with the phenotypic method.

The mutations that occur spontaneously are then selected by the drug. When resistant mutants constitute an essential part of the *H pylori* population, given that the mutations do not have any impact on the bacterial fitness, the number of mutants in the population do not decrease with time (31).

RESISTANCE TO OTHER ANTIBIOTICS

Amoxicillin

Amoxicillin is the second antibiotic commonly used to treat *H pylori* infection. Fortunately, resistance to this drug appears to be very limited. Indeed, Dore et al (32) reported the existence of *H pylori* strains tolerant to amoxicillin (ie, the ratio of the minimal bactericidal concentration to the minimal inhibitory concentration [MIC] is greater than 32 mg/L). This tolerance was not always observed after freezing of the strains. The authors hypothesized that it was due to a lack of a certain penicillin binding protein (PBP), named PBP-4 (33). The clinical significance of this phenomenon is not clear.

Another resistance mechanism was described in a strain isolated in the Netherlands from a patient who had received multiple courses of amoxicillin for respiratory tract infections. The moderate level of resistance (8 mg/L) was transferable (34).

What's new? By sequencing, Gerrits et al (35) have shown that a single point mutation on the *pbp1A* gene concerning a serine 414-arginine substitution was able to increase the MIC of susceptible strains to 1 mg/L after transformation.

Another study using an *H pylori* strain selected by culturing an amoxicillin-susceptible strain in increasingly higher concentrations of amoxicillin resulted in an MIC increase from 0.02 mg/L to 15 mg/L. The resistant strain showed mutations on *pbp1* involving four amino acids. However, the transformation of a susceptible strain with the mutated *pbp1* gene rendered these strains only moderately resistant, suggesting that mutations in other genes may be involved (36).

In a similar experiment, in which the strain MIC increased from 0.03 mg/L to 4.8 mg/L, the resistant strain exhibited a sig-

nificant decrease in PBP1 binding, and a decrease in the uptake of the labelled amoxicillin (37).

Resistance to amoxicillin is still seldom found and, so, its impact on the clinical outcome of treatment is still not known. However, it is expected that an increase in the future due to selective pressure will be seen.

No molecular method is currently available to detect this resistance. The best method consists of measuring the strain MIC, and this is achieved using the E-test.

Metronidazole

In recent guidelines, metronidazole was recommended in second-line therapies, and especially in quadruple therapy.

The prevalence of *H pylori* resistance to metronidazole is quite high. In Europe, the previously reported study indicated a global resistance rate of 33% with a higher rate in southern Europe (40.8%) (6). A similar resistance rate is found in the United States (39%) (9), Taiwan (32%) and Australia (32%), but not in Japan (4.1%), because this drug is seldom used there.

The impact of metronidazole resistance on the clinical outcome of *H pylori* eradication is relatively modest: the eradication rate decreases by 20% when the strain is resistant rather than susceptible (38).

The mechanism of metronidazole resistance is not yet fully understood. Metronidazole must be reduced to exert its damage on bacterial DNA. The main enzyme involved is an oxygen-insensitive nitroreductase *rdxA* as reported by Goodwin et al (39). Mutation in the *rdxA* gene can alter its nitroreductase activity and render the organism resistant. Several mutations may be involved and, furthermore, all *rdxA* mutations are not associated with a resistant phenotype. In addition, the RdxA protein may be produced but may not express its activity. Another gene, *frxA*, can modulate the expression of *rdxA*, but this is a topic of controversy as was highlighted in a recent review (40). Some authors believe that *frxA* mutations may also lead to a resistant phenotype (41), while others see *frxA* only as a molecule which enhances the level of resistance of *rdxA* mutants (42).

What's new? The difficulty in performing *H pylori* susceptibility testing for metronidazole has been reported many times. In a European study comparing the E-test with the agar dilution method used in four laboratories, major errors for categorizing the susceptibility to metronidazole were found in 32.5% of the cases, indicating an unacceptable intertest variability (43).

The optimal method may, in fact, be to look for the RdxA protein by immunoblot as proposed by Latham et al (44). They obtained a positive result in 100% of 17 strains categorized as positive, but only in 7.5% categorized as negative.

Tetracycline

Tetracycline is also used in second-line treatments, and especially in the quadruple therapy.

The first resistant strain was isolated in Australia, but the mechanism was not known at that time (45). Subsequently, other reports have been published, but this resistance remains rare except in Korea, where a 5.3% (46) rate was reported and resistance could be transferred (47). However, the impact on clinical outcome has not yet been documented.

What's new? Importantly, the mechanism has been described independently by two groups. Trieber and Taylor (48), studying the first reported Australian resistant strain, found a triple mutation AGA965-967TTC in the *16S rRNA* gene, in a region adjacent to the tetracycline binding site. Gerrits et al (49), using another resistant strain, observed that the same triple mutation was present, indicating a high probability of a causal association.

TABLE 2
Genes concerned by point mutations or other genetic events leading to antibiotic resistance in *Helicobacter pylori*, and frequency of resistance

Antibiotic group	Genes concerned	Frequency of resistance (%)
Macrolides	*23S rRNA*	0-20
Metronidazole	*rdxA, frxA*	10-90
Quinolones	*gyrA*	0-10
Rifamycins	*rpoB*	0-5
Amoxicillin	*PBP1A*	Few cases described
Tetracycline	*16S rRNA*	Few cases described

Rifamycins

Among the rifamycins, only rifabutin has been proposed as a rescue therapy. No resistance has been reported in vivo, but resistance can be selected in vitro. Heep et al (50) reported that resistant cases were due to point mutations in the 69 base pair region of the *rpoB* gene; this gene codes for the beta subunit of the RNA polymerase that is the target of rifamycins.

Quinolones

Recently, several studies have shown a good efficacy for cerfloxacin. The rate of resistance is still low (3% in France), but may be more important in countries with a high consumption of quinolones, such as Portugal. In a study dating back to 1995, it was shown that resistance, which occurs easily, is associated with three point mutations in the *gyrA* gene (51).

IMPLICATIONS

It is interesting to note that all of the known mechanisms of resistance have now been described for antibiotics commonly used for *H pylori* eradication. They are all related to point mutations of genes present on the bacterial chromosome (Table 2), making genetic transfer unlikely, except possibly by transformation. One consequence is the slow increase in resistance because it only concerns the descendants of the mutated bacteria and is not a horizontal transfer. Nevertheless, when resistance to macrolides reaches 20%, as it does in France, the strategy of empiric use of clarithromycin must be challenged. We now have a method that allows a 2 h diagnosis of *H pylori* infection and of its susceptibility to macrolides, making it worthwhile to perform susceptibility testing to provide a tailored choice of treatment, rather than an empirical clarithromycin-based treatment.

For the detection of metronidazole resistance, a new testing method based on the immunoblot may become the method of choice because molecular methods cannot be applied and standard methods are notoriously unreliable.

In summary, progress in eradication therapy will be a result of the progress in susceptibility testing, and we are now close to revising the guidelines to propose susceptibility testing before the first treatment is attempted, rather than waiting for a failure of cure.

REFERENCES

1. Report of the Digestive Health Initiative International Update Conference on *Helicobacter pylori*. Gastroenterology 1997;113(Suppl):S4-8.
2. Lam SK, Talley NJ. Report of the 1997 Asia Pacific Consensus Conference on the management of *Helicobacter pylori* infection. J Gastroenterol Hepatol 1998;13:1-12.
3. Coehlo LG, Leon-Barua R, Quigley EMM. Latin-American consensus conference on *Helicobacter pylori* infection. Am J Gastroenterol 2000;95:2688-91.
4. Malfertheiner P, Mégraud F, O'Morain C, et al. Current concepts in the management of *Helicobacter pylori* infection – the Maastricht 2-2000 Consensus Report. Aliment Pharmacol Ther 2002;16:167-80.
5. Wermeille J, Cunningham M, Dederding JP, et al. Failure of *Helicobacter pylori* eradication: Is poor compliance the main cause? Gastroenterol Clin Biol 2002;26:216-9.
6. Glupczynski Y, Mégraud F, Lopez-Brea M, Andersen LP. European multicenter survey of in vitro antimicrobial resistance in *Helicobacter pylori*. Eur J Clin Microbiol Infect Dis 2000;11:820-3.
7. Seppälä H, Klaukka T, Vuopio-Varkila J, et al. The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland. N Engl J Med 1997;337:441-6.
8. Cars O, Mölstad S, Melander Z. Variation in antibiotic use in the European Union. Lancet 2001;357:1851-3.
9. Osato MS, Reddy R, Reddy SG, Penland RL, Malaty HM, Graham DY. Pattern of primary resistance of *Helicobacter pylori* to metronidazole or clarithromycin in the United States. Arch Intern Med 2001;161:1217-20.
10. Laine L, Fennerty MB, Osato M, et al. Esomeprazole-based *Helicobacter pylori* eradication therapy and the effect of antibiotic resistance: Results of three US multicenter, double-blind trials. Am J Gastroenterol 2000;95:3393-8.
11. Fallone CA. Epidemiology of the antibiotic resistance of *Helicobacter pylori* in Canada. Can J Gastroenterol 2000;14:879-82.
12. Houben MHMG, van de Beek D, Hensen EF, De Craen JM, Rauws EAJ, Tytgat GNJ. A systematic review of *Helicobacter pylori* eradication therapy – The impact of antimicrobial resistance on eradication rates. Aliment Pharmacol Ther 1999;13:1047-56.
13. Broutet N, Tchamgoué S, Pereira E, Lamouliatte H, Salamon R, Mégraud F. Risk factors for failure of *Helicobacter pylori* therapy – Results of an individual data analysis of 2,751 patients. Aliment Pharmacol Ther 2003;17:99-109.
14. Mégraud F. *Helicobacter pylori* and macrolides. In: Macrolide Antibiotics, Schönfeld W, Kirst HA, eds. Birkhäuser Verlag: Berlin, 2002:243-60.
15. Versalovic J, Shortridge D, Kliber K, et al. Mutations in 23S rRNA are associated with clarithromycin resistance in *Helicobacter pylori*. Antimicrob Agents Chemother 1996;40:477-80.
16. Occhialini A, Urdaci M, Doucet-Populaire F, Bébéar CM, Lamouliatte H, Mégraud F. Macrolide resistance in *Helicobacter pylori*: Rapid detection of point mutations and assays of macrolide binding to ribosomes. Antimicrob Agents Chemother 1997;41:2724-8.
17. Stone GG, Shortridge D, Versalovic J, et al. A PCR-oligonucleotide ligation assay to determine the prevalence of 23S rRNA gene mutations in clarithromycin-resistant *Helicobacter pylori*. Antimicrob Agents Chemother 1997;41:712-4.
18. Pina M, Occhialini A, Monteiro L, Doermann HP, Mégraud F. Detection of point mutations associated with resistance of *Helicobacter pylori* to clarithromycin by hybridization in liquid phase. J Clin Microbiol 1998;36:3285-90.
19. Marais A, Monteiro L, Occhialini A, Pina M, Lamouliatte H, Mégraud F. Direct detection of *Helicobacter pylori* resistance to macrolides by a polymerase chain reaction/DNA enzyme immunoassay in gastric biopsy specimens. Gut 1999;44:463-7.
20. Maeda S, Yoshida H, Matsunaga H, et al. Detection of clarithromycin-resistant *H pylori* strains by a preferential homoduplex formation assay. J Clin Microbiol 2000;38:210-4.
21. van Doorn LJ, Debets-Ossenkopp YJ, Marais A, et al. Rapid detection, by PCR and reverse hybridization, of mutations in the *Helicobacter pylori* 23S rRNA gene, associated with macrolide resistance. Antimicrob Agents Chemother 1999;43:1779-82.
22. Alarcón T, Domingo D, Prieto N, Lopez-Brea M. PCR using 3′-mismatched primers to detect A2142C mutation in 23S rRNA conferring resistance to clarithromycin in *Helicobacter pylori* clinical isolates. J Clin Microbiol 2000;38:923-5.
23. Scarpellini P, Carrera P, Cavallero A, et al. Direct detection of *Helicobacter pylori* mutations associated with macrolide resistance in gastric biopsy material taken from human immunodeficiency virus-infected subjects. J Clin Microbiol 2002;40:2234-7.
24. Trebesius K, Panthel K, Strobel S, et al. Rapid and specific detection of *Helicobacter pylori* macrolide resistance in gastric tissue by fluorescent in situ hybridisation. Gut 2000;46:608-14.
25. NCCLS. Performance standard s for antimicrobial susceptibility testing. VIth Informational Supplement. 1999;M100S9:19: 1.
26. Grignon B, Tankovic J, Mégraud F, et al. Validation of diffusion methods for macrolide susceptibility testing of *Helicobacter pylori*. Microb Drug Resist 2002;8:61-6.
27. Ménard A, Santos A, Mégraud F, Oleastro M. 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 2002;46:1156-7.
28. Chisholm SA, Owen RJ, Teare EL, Saverymuttu S. PCR-based diagnosis of *Helicobacter pylori*: Infection and real-time deterination of clarithromycin resistance directly from human gastric biopsy samples. J Clin Microbiol 2001;39:1217-20.
29. Matsumura M, Hikiba Y, Ogura K, et al. Rapid detection of mutations in the 23S rRNA gene of *Helicobacter pylori* that confers resistance to clarithromycin treatment to the bacterium. J Clin Microbiol 2001;39:691-5.
30. Oleastro M, Ménard A, Santos A, et al. Rapid and accurate detection of point mutations conferring resistance to clarithromycin in *Helicobacter pylori* by real-time PCR. J Clin Microbiol 2003;41:397-402.
31. Hulten K, Gibreel A, Sköld O, Engstrand L. Macrolide resistance in *Helicobacter pylori*: Mechanism and stability in strains from clarithromycin-treated patients. Antimicrob Agents Chemother 1997;41:2550-3.
32. Dore MP, Osato MS, Realdi G, Mura I, Graham DY, Sepulveda AR. Amoxycillin tolerance in *Helicobacter pylori*. J Antimicrob Chemother 1999;43:47-54.
33. Dore MP, Graham DY, Sepulveda AR. Different penicillin-binding protein profiles in amoxicillin-resistant *Helicobacter pylori*. Helicobacter 1999;4:154-61.
34. van Zwet AA, Vandenbroucke-Grauls CM, Thijs JC, van der Wouden EJ, Gerrits MM, Kusters JG. Stable amoxicillin resistance in *Helicobacter pylori*. Lancet 1998;352:1595.
35. Gerrits MM, Schuijffel D, van Zwet AA, Kuipers EJ, Vandenbroucke-Grauls CM, Kusters JG. Alterations in penicillin-binding protein 1A confer resistance to betalactam antibiotics in *Helicobacter pylori*. Antimicrob Agents Chemother 2002;46:2229-33.
36. Paul R, Postius S, Melchers K, Schafer KP. Mutations of the *Helicobacter pylori* genes *rdxA* and *pbp1* cause resistance against metronidazole and amoxicillin. Antimicrob Agents Chemother 2001;45:962-5.
37. DeLoney CR, Schiller NL. Characterization of an in vitro-selected amoxicillin-resistant strain of *Helicobacter pylori*. Antimicrob Agents Chemother 2000;44:3368-73.
38. Mégraud F, Lehn N, Lind R, et al. Antimicrobial susceptibility testing of *Helicobacter pylori* in a large multicenter trial. The MACH 2 study. Antimicrob Agents Chemother 1999;43:2747-52.
39. Goodwin A, Kersulyte D, Sisson G, Veldhuysen van Zanten SJO, Berg D, Hoffman PS. Metronidazole resistance in *Helicobacter pylori* is due to null mutations in a gene (*rdxA*) that encodes an oxygen-insensitive NADPH nitroreductase. Mol Microbiol 1998;28:383-93.
40. Mendz G, Mégraud F. Is the molecular basis of metronidazole resistance in microaerophilic organisms understood? Trends Microbiol 2002;10:370-5.
41. Kwon DH, Kato M, El-Zaatari FA, Osato MS, Graham DY. Frame-shift mutations in NAD(P)H flavin oxidoresuctase encoding gene (*frxA*) from metronidazole resistant *Helicobacter pylori* ATCC43505 and its involvement in metronidazole resistance. FEMS Microbiol Lett 2000;118:197-202.
42. Jeong JY, Mukhopadhyay AK, Akada JK, Dailidiene D, Hoffman PS, Berg DE. Roles of FrxA and RdxA nitroreductases of *Helicobacter pylori* in susceptibility and resistance to metronidazole. J Bacteriol 2001;183:5155-62.
43. Glupczynski Y, Broutet N, Cantagrel A, et al. Comparison of the E test and agar dilution method for antimicrobial susceptibility testing of *Helicobacter pylori*. Eur J Clin Microbiol Infect Dis 2002;21:549-52.
44. Latham SR, Owen RJ, Elviss NC, Labigne A, Jenks PJ. Differentiation of metronidazole-sensitive and -resistant clinical isolates of *Helicobacter pylori* by immunoblotting with antisera to the RdxA protein. J Clin Microbiol 2001;39:3052-5.
45. Midolo PD, Korman MG, Turnidge JD, Lambert JR. *Helicobacter pylori* resistance to tetracycline. Lancet 1996;347:1194-5.
46. Kim JJ, Reddy R, Lee M, et al. Analysis of metronidazole, clarithromycin and tetracycline resistance of *Helicobacter pylori* isolates from Korea. J Antimicrob Chemother 2001;47:459-61.
47. Kwon DH, Kim JJ, Lee M, et al. Isolation and characterization of tetracycline-resistant clinical isolates of *Helicobacter pylori*. Antimicrob Agents Chemother 2000;44:3203-5.
48. Trieber AA, Taylor DE. Mutations in the 16S rRNA genes of *Helicobacter pylori* mediate resistance to tetracycline. J Bacteriol 2002;184:2131-40.
49. Gerrits MM, de Zoete MR, Arents NLA, Kuipers EJ, Kusters JG. 16S rRNA mutation-mediated tetracycline resistance in *Helicobacter pylori*. Antimicrob Agents Chemother 2002;46:2996-3000.
50. Heep M, Odenbreit S, Beck D, et al. Mutations at four distinct regions of the *rpoB* gene can reduce the susceptibility of *Helicobacter pylori* to rifamycins. Antimicrob Agents Chemother 2000;44:1713-5.
51. Moore RA, Beckthold B, Wong S, Kureishi A, Bryan LE. Nucleotide sequence of the gyrA gene and characterization of ciprofloxacin-resistant mutants of *Helicobacter pylori*. Antimicrob Agents Chemother 1995;39:107-11.