# Antibiotic susceptibility patterns in *Helicobacter pylori* strains from patients with upper gastrointestinal pathology in western Nigeria

K. S. OYEDEJI[*], S. I. SMITH[*], A. O. COKER[†] and A. O. ARIGBABU[‡]

[*]*Nigerian Institute of Medical Research, Yaba, Lagos;* [†]*College of Medicine, University of Lagos, Idi-Araba, Lagos; and* [‡]*Endoscopy Unit, Obafemi Awolowo University Teaching Hospital, Ife, Nigeria*

*Accepted: 9 June 2008*

ABSTRACT

A total of 186 *Helicobacter pylori* isolates and 532 gastric biopsies recovered from 532 patients with varying degrees of gastroduodenal pathology are subjected to *in vitro* antibiotic susceptibility testing using the disc-diffusion method, Etest (MIC breakpoints) and molecular testing using the polymerase chain reaction (PCR). In the isolates studied, antibiotic resistance was as follows: piperacillin (72%), amoxicillin (66%), erythromycin (78%), tetracycline (100%) and metronidazole (95%). All isolates were sensitive to ofloxacin, ciprofloxacin and norfloxacin. None of the 245 amplicons (positive for *H. pylori*) from the biopsies were digested with the *Bbs*1 and *Bsa*1 restriction enzyme used in the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) technique, showing sensitivity to clarithromycin. However, a 238 bp fragment from *H. pylori* chromosomal DNA (corresponding to the quinolone resistance determining region [QRDR]) of the *gyr*A gene was amplified successfully. Twelve (4.9%) of the 245 strains studied had the described mutation at position 91, from asparagine (Asn) to glycine (Gly). The study showed that all the *H. pylori* strains were sensitive to clarithromycin and ciprofloxacin. It also highlighted PCR as a potential tool for faster diagnosis and determination of antibiotic susceptibility (within 24 h) of *H. pylori* from biopsies and/or isolates recovered from peptic ulcer and gastritis patients.

KEY WORDS: Antibiotics.
Helicobacter pylori.
Polymerase chain reaction.
Polymorphism, restriction fragment length.
Quinolones.

## Introduction

Eradication of *Helicobacter pylori* is the most effective means to cure peptic ulcer disease and prevent possible recurrent episodes. Common treatment regimens include a proton-pump inhibitor and two antimicrobial agents (amoxicillin, clarithromycin, metronidazole or tetracycline).

*H. pylori* generally develops resistance to many antibiotics.[1,2] Remarkably, the known mechanisms of antibiotic resistance in *H. pylori* are not plasmid-mediated but are due to mutations in chromosomal genes, even though plasmids can be detected in half of the isolates.[3] In *H. pylori*, the genetic determinants for resistance to antibiotics such as clarithromycin, metronidazole, ciprofloxacin and rifampin have been determined.[4]

Clarithromycin resistance is associated with mutations in the 23S ribosomal RNA (rRNA) gene,[5,6] which inhibits the binding of clarithromycin to the ribosome, while rifampin resistance results from mutations in the *rpo*B gene, encoding the B-subunit of RNA polymerase.[7] In metronidazole resistance, although several different mechanisms may exist, the predominant determinant is mutational inactivation of the *rdx*A gene that encodes an oxygen-sensitive NADPH nitroreductase.[8]

Quinolones exert antimicrobial activity on *H. pylori* by inhibiting the enzyme DNA gyrase.[9] The region of the mutation for resistance in quinolones has been termed the quinolone resistance determining region (QRDR).[10] Resistance to quinolones by *H. pylori* has been attributed to specific mutations in the *gyr*A gene,[11] which has been cloned and sequenced. The sequencing of the amplification product from ciprofloxacin-resistant mutants of *H. pylori* reveals four classes of mutations with substitutions at amino acid 87 (Asn→Lys), 88 (Ala→Val) and 91 (Asn→Gly, Asp→Tyr) and a double substitution at amino acids 91 and 97 (Ala→Val).[1]

However, indications for treatment of *H. pylori* have been extended[12] and the probability of emergence of resistant strains increases.[13] The successful treatment of any infection lies with the results of *in vitro* susceptibility testing. In *H. pylori* infection, the progression of antibiotic resistance requires a faster approach in performing the susceptibility testing using modern molecular techniques that combine the direct detection of *H. pylori* infection on the biopsy as well as detection of antibiotic resistance.[3]

This study compares the antimicrobial susceptibility patterns of *H. pylori* strains isolated from dyspeptic patients in western Nigeria using conventional and molecular techniques.

*Correspondence to: Dr. Kolawole Solomon Oyedeji*
*Molecular Biology and Biotechnology Division,*
*Nigerian Institute of Medical Research, Yaba,*
*PMB 2013, 6 Edmond Crescent, Yaba, Lagos, Nigeria*
*Email: kolaremi95@yahoo.com*

## Materials and methods

*Study population*
Biopsies collected from 532 patients presenting with varying degrees of gastroduodenal pathology from three hospitals in western Nigeria were studied. All patients provided informed consent and ethical approval was given by the ethical review committee of Lagos University Teaching Hospital, Lagos, and Obafemi Awolowo Teaching Hospital Complex, Ile-Ife, Nigeria.

*Sample processing*
Primary isolation of the organism was performed on Dent's medium[14] consisting of Columbia agar (7%), laked horse blood and Dent's supplement (10 mg vancomycin, 5 mg trimethoprim, 5 mg cefsulodin and 5 mg amphotericin B per litre) (Oxoid). From the 532 biopsies cultured, 186 isolates were obtained for the disc-diffusion and Etest methods of antibiotic susceptibility testing.

*Disc-diffusion method*
The method of Baeur *et al.*[15] was employed. The antibiotic discs used were amoxicillin (10 µg), erythromycin (15 µg), piperacillin (100 µg), imipenem (10 µg), gentamicin (10 µg), tetracycline (10 µg), norfloxacin (10 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), kanamycin (30 µg), metronidazole (50 µg) and cefuroxime (10 µg).

The growth of *H. pylori* from the culture plate was used to produce 2 mL 0.5 McFarland standards. When the growth was not sufficient to produce this, the growth was subcultured and incubated for a further three days before performing resistance tests.

For the resistance test, plates were flooded with approximately 2 mL of a 0.5 McFarland suspension ($10^7$ colony-forming units [cfu]/mL). Excess fluid was removed immediately[16] and the plates were incubated at 37°C in a candle extinction jar or anaerobic gas pack and the results were read after 24 h.

Inhibition zone diameters of ≤20 mm (amoxicillin), ≤12 mm (gentamicin and macrolides), ≤14 mm (tetracycline), ≤28 mm (metronidazole) and ≤8 mm (quinolones) were regarded as indicating resistance.[17]

*H. pylori* control strains used were CCUG 38770, CCUG 38771 and CCUG 38772 (German reference strains for *H. pylori*).

*Etest method*
One plate was used for each Etest strip. The plates were allowed to dry for 10 min and the strips were kept on the bench for 10–15 min to attain room temperature. The antibiotics tested were metronidazole, clarithromycin, amoxicillin, tetracycline and ciprofloxacin.

Colonies from pure cultures of *H. pylori* were transferred using a sterile loop into a sterile tube containing 4–5 mL 0.9 % NaCl to give the opacity of a McFarland Standard 2 (0.5). The solution was flooded over the sensitivity plate (Columbia agar) and excess fluid was removed with a pipette. After drying for 15–20 min, an Etest strip was placed on each plate and then incubated under microaerophilic conditions at 37°C for 24–36 h.

Breakpoints for minimum inhibitory concentration (MIC)[18] of the antibiotics were: metronidazole (8 µg/mL [sensitive], >8 µg/mL [resistant]), clarithromycin (≤0.25 µg/ mL [sensitive], ≥1 µg/mL [resistant]), amoxicillin (<0.5 µg/mL [sensitive], ≥0.5 µg/mL [resistant), tetracycline (<4 µg/ mL [sensitive], ≥4 µg/mL [resistant]) and ciprofloxacin (<1 µg/mL [sensitive], ≥1 µg/mL [resistant]).

*H. pylori* control strains used were CCUG 38770, CCUG 38771 and CCUG 38772 (German reference strains for *H. pylori*).

**Table 1.** Antibiotic susceptibility patterns of *H. pylori* strains isolated from peptic ulcer and gastritis patients.

| Antibiotic | Resistant (%) (*n*=186) | Zone size (mm) Isolates | Control strains |
|---|---|---|---|
| Amoxicillin (10 µg) | 123 (66) | 6.4 | 45 |
| Erythromycin (15 µg) | 145 (78) | 6.4 | 28 |
| Piperacillin (100 µg) | 134 (72) | 6.4–7.6 | 28 |
| Imipenem (10 µg) | 0 (0) | 15–30 | 32 |
| Gentamicin (10 µg) | 139 (75) | 15–18 | 24 |
| Tetracycline (10 µg) | 186 (100) | 6.4 | 24 |
| Norfloxacin (10 µg) | 0 (0) | 18–30 | 32 |
| Ofloxacin (5 µg) | 0 (0) | 18–30 | 30 |
| Ciprofloxacin (5 µg) | 0 (0) | 18–30 | 34 |
| Kanamycin (30 µg) | 139 (75) | 15–18 | 24 |
| Metronidazole (50 µg) | 177 (95) | 6.4–7.6 | 30 |
| Cefuroxime (10 µg) | 138 (75) | 22–30 | 34 |

*DNA extraction from gastric biopsies*
Biopsies were ground and centrifuged for 5 min at 10,000 x*g*. The pellet produced was resuspended in 300-µL extraction buffer (20 mmol/L Tris-HCl [pH 8.0], 0.5% Tween 20) and proteinase K was added at a final concentration of 0.5 mg/mL. The mixture was incubated at 56°C for 1 h. Finally, the enzyme was inactivated by boiling for 10 min. The PCR method was performed using the *vac*A, *cag*A and *ure*I primers to ascertain that the extracted DNA was from *H. pylori*.

*Amplification of the QRDR*
The QRDR was amplified by PCR using the primers (5′ AATT AGGCC TTA – CTTCCA AAG TCG CTT ACA [forward] and 5′ TCT TCA CTCGCC TTA GTC ATTC TG GC [reverse]) corresponding to an amplification product of 238 bp. Thermocycling parameters used were 93°C for 2 min and 35 cycles of 93°C for 10 sec, 49°C for 10 sec, 72°C for 1.5 min, one cycle of 72°C for 10 min and a 10-min hold at 8°C. Amplification was performed in a 50 µL reaction volume. Reaction products were visualised by running 10 µL reaction mixture on a 1.2% agarose gel, stained with ethidium bromide.

The reaction mixture for the PCR sequencing of the amplified DNA from the various strains of *H. pylori* comprised 2 µL PCR product, 1.3 µL primer 1 (2.5 µmol/L), 4 µL reaction mix and 12.7 µL $H_20$. Thermocycling parameters were one cycle at 40°C for 2 min, 93°C for 3 min, 25 cycles at 96°C for 10 sec, 50°C for 5 sec, 60°C for 4 min, and one cycle at 4°C for 10 min.

The resulting products were washed with isopropanol for 30 min. The pellets were dried for 1 min and the products were sequenced and later analysed.

***PCR–RFLP technique***

The 23S fragment of *H. pylori* DNA was obtained by amplification of the extracted DNA with primers HPYS (5″ – AGG TTAAGA GGA TGC GTC AGTC – 3′, nucleotides 2302–2323) and HPYA (5′ ACT GCT AAT GGG AAT ATC ATG CG – 3′, nucleotides 2568–2546) (GenBank accession number U27270). Amplification of the DNA was performed in a final volume of 50 µL containing 3 µL DNA and 47 µL master mix (5 µL 10x PCR buffer [Euro Bios], 1.5 µL MgCl [50 mmol/L], 0.4 µL dNTPs, 0.5 µL each primer [HPYS and HPYA], 0.2 µL *Thermus aquaticus* [*Taq*] polymerase, [Euro Bios] and 38.9 µL water). Thermocycling was one cycle at 94°C for 5 min, 39 cycles at 94°C for 1 min, 55°C for 1 min, 72°C for 1 min and a final elongation step at 72°C for 8 min. The amplified 267-bp fragment was visualised after electrophoresis on a 1.5% agarose gel stained with ethidium bromide.

Digestion of the 23S fragment with the restriction enzymes *Bbs*1 and *Bsa*1 permitted the discrimination between two mutant genotypes (A2143G and A2144G, respectively). Digestions was performed in a final volume of 15 µL containing 5 µL amplicons, 1.5 µL buffer 2 (10x) and buffer 4 (10x) for *Bbs*1 and *Bsa*1, respectively, 7.5 µL water and 1.0 µL each enzyme. *Bbs*1 was incubated at 37°C overnight, while *Bsa*1 was incubated at 50°C overnight. The restriction products were visualised after electrophoresis on a 2.5% agarose gel stained with ethidium bromide.[19]

## Results

The susceptibility patterns of the 186 isolates are shown in Tables 1 and 2.

Of the 532 samples tested, 245 were positive with the Hp 23–1 and Hp 23-2 primers, indicating that 46.1% of the patients had *H. pylori* DNA. When submitted to the restriction enzymes *Bbs*1 and *Bsa*1, none of the 245 amplicons were digested, indicating the absence of mutations associated with macrolide resistance.

A 238 bp DNA fragment from *H. pylori* chromosomal DNA was amplified successfully from 245 of the 532 biopsies. This corresponded to the QRDR of the *gyr*A gene of *H. pylori*.

Results of quinolone resistance screening are shown on Table 3.

**Table 2.** Results of E- test (MIC breakpoints) of *H. pylori* isolates to the different antibiotics.

| Antibiotic | Number of isolates | MIC breakpoints | Percentage resistance |
|---|---|---|---|
| Amoxicillin | 123 (66.1%) | 4 µg/mL | 100% |
| <0.5 µg/mL (S) | 42 (22.6%) | 32 µg/mL | 100% |
| | 11 (5.9%) | 16 µg/mL | 100% |
| | 10 (5.4%) | 8 µg/mL | 100% |
| Tetracycline | 123 (66.1%) | 128 µg/mL | 100% |
| <4 µg/mL (S) | 42 (22.6%) | 256 µg/mL | 100% |
| | 11 (5.9%) | 16 µg/mL | 100% |
| | 10 (5.4%) | 32 µg/mL | 100% |
| Metronidazole | 123 (66.1%) | >256 µg/mL | 100% |
| <8 µg/mL (S) | 42 (22.6%) | 32 µg/mL | 100% |
| | 11 (5.9%) | 64 µg/mL | 100% |
| | 10 (5.4%) | 16 µg/mL | 100% |
| Ciprofloxacin | 123 (66.1%) | 0.019 µg/mL | 0% |
| <1 µg/mL (S) | 42 (22.6%) | 0.019 µg/mL | 0% |
| | 11 (5.9%) | 0.019 µg/mL | 0% |
| | 10 (5.4%) | 0.38 µg/mL | 0% |
| Clarithromycin | 123 (66.1%) | 0.016 µg/mL | 0% |
| <0.25 µg/mL (S) | 42 (22.6%) | 0.016 µg/mL | 0% |
| | 11 (5.9%) | 0.023 µg/mL | 0% |
| | 10 (5.4%) | 0.023 µg/mL | 0% |

**Table 3.** Detected mutations in QRDR amplified from *H. pylori.*

| Mutation | Number of strains ($n$=245) | Quinolone resistance |
|---|---|---|
| 87 Asn → Lys | 0 | No |
| 91 Asp → Gly | 12 (4.9 %) | Yes |
| 91 Asp → Asn | 0 | No |
| 91 Asp → Tyr | 0 | No |
| 91 and 97 Ala → Val | 0 | No |
| 87 Asn → Thr | 5 (2.0%) | Yes |
| Total | 17 (6.9%) | |

## Discussion

This study has shown that all the *H. pylori* strains isolated from the patients studied were resistant to tetracycline and metronidazole, while the majority of the isolates were resistant to amoxicillin. Unfortunately, these drugs form the basis of treatment for *H. pylori* infection worldwide.[20–22]

High amoxicillin resistance in *H. pylori* has been reported in western Nigeria.[2] Typical drug combinations in western Nigeria comprise amoxicillin, metronidazole, omeprazole (proton pump inhibitor) or bismuth. Treatment failure, however, has been reported in some patients, and there is also the attendant problem of patients not returning to clinic, mostly because of the cost of the drugs. Thus, complete treatment of the infection is difficult.

In a study conducted in the middle belt of Nigeria, 100% susceptibility was recorded for amoxicillin, 89.0% for tetracycline, 87.3% for clarithromycin and 60% for metronidazole;[23] however, the results of the present study differ. One possible reason for the observed high resistance rate for amoxicillin, tetracycline and metronidazole is the fact that most medical practitioners in western Nigeria prescribe these drugs for other ailments (e.g., metronidazole for parasitic infections and urinary tract infections), amoxicillin is sold on the streets and is used for treatment of typhoid fever, and tetracycline is used indiscriminately for any slight stomach upset.

Furthermore, some workers suggest that antibiotic resistance or treatment failure is due to poor patient compliance as a result of side effects, with approximately 10% of patients discontinuing use of the drugs.[24,25] This could partly explain the resistance patterns observed in this study, as inadequate dosage or non-compliance may cause the pathogen to develop resistant mutants as an adaptive mechanism against future treatment.[26,27]

Clarithromycin has been reported as the most active agent among the macrolides tested for the treatment of *H. pylori* infections,[28] as seen also in the present study. It is also

apparent that there is no emerging resistance and cross-reaction to this group of antibiotics.

The results of the present study indicate that molecular methods have the potential to be used to detect *H. pylori* from biopsies and its eventual resistance to macrolides and other antibiotics. It also indicates that *H. pylori* is still fully susceptible to clarithromycin in Nigeria. Therefore, it is possible to detect by PCR–RFLP the three most frequent point mutations of the 23S rRNA gene that confer clarithromycin resistance in *H. pylori*. This method can be applied to *H. pylori* strains and also to gastric biopsy samples, allowing the detection of the three genotypes with one PCR sample.

This study shows that the use of quinolones can be continued in Nigeria. Only 12 (4.9%) of the 245 *H. pylori* isolates studied had the described mutation at position 91 (Asn→Gly). These findings also demonstrate that resistance to ciprofloxacin is due primarily to alterations in a relatively small portion of the *H. pylori gyr*A gene.

A further interesting result from the sequencing is a case of an undescribed mutation (87 Asn→Thr); however, this could not be confirmed as no transformation was performed for this mutation. Five (2%) of the 245 isolates showed this amino acid exchange.

The reliability of methods for determining antibiotic resistance in *H. pylori* depends on factors such as cost, expertise and time of reading results. Comparison of the techniques evaluated in this study shows that molecular resistance testing is faster and more reliable. However, the disc-diffusion method is cheap and reliable for use in developing countries such as Nigeria, where the Etest and MIC methods are too expensive to be performed in a routine laboratory. □

## References


1 Moore RA, Beckhold,B, Wong S, Kureishi A, Bryan LE. Nucleotide sequence of the *gyr*A gene and characterization of the ciprofloxacin-resistant mutants of *Helicobacter pylori*. *Antimicrob Agents Chemother* 1995; **39**: 107–11.

2 Smith SI, Oyedeji KS, Arigbabu, AO, Atimomo CE, Coker AO. High amoxycillin resistance in *Helicobacter pylori* isolated from gastritis and peptic ulcer patients in western Nigeria. *J Gastroenterol* 2001; **36**: 67–8.

3 Rautelin H, Lehours P, Megraud F. Diagnosis of *Helicobacter pylori* infection. *Helicobacter* 2003; **8**: 13–20.

4 Megraud F. Epidemiology and mechanism of antibiotic resistance in *Helicobacter pylori*. *Gastroenterology* 1998: **115**: 1278–82.

5 Versalovic H, Shortridge D, Kibler K *et al*. Mutations in 23S rRNA are associated with clarithromycin resistance in *Helicobacter pylori. Antimicrob Agents Chemother* 1996 ; **40**: 477–80.

6 Taylor DE, Ge Z, Purych, D, Lo T, Hiratsuka K. Cloning and sequence analysis of the two copies of 23S rRNA genes from *Helicobacter pylori* and association of clarithromycin resistance with 23S rRNA mutations. *Antimicrob Agents Chemother* 1997; **41**: 2621–8.

7 Heep M, Beck D, Bayerdorffer E, Lehn N. Rifampin and rifabutin resistance mechanism in *Helicobacter pylori. Antimicrob Agents Chemother* 1999; **43**: 1497–9.

8 Tankovic J, Lamarque D, Delchier JC, Sonssy CJ, Labigne A, Jeuks PJ. Frequent association between alteration of the *rdx*A gene and metronidazole resistance in French and North African isolates of *Helicobacter pylori. Antimicrob Agents Chemother* 2000; **44**: 608–13.

9 Reece RJ, Maxwell A. DNA gyrase structure and function. *Crit Rev Biochem Mol Biol* 1991; **26** (3–4): 335–75.

10 Yoshida H, Bogaki M, Nakamurh M, Sakamura S. Quinolone resistance-determining region in the DNA gyrase *gyr*A gene of *Escherichia coli. Antimicrob Agents Chemother* 1990; **34**: 1271–2.

11 Wang Y. Hung, WM, Taylor DE. Cloning and nucleotide sequence of the *Campylobacter pylori gyr*A gene and characterization of quinolone resistance mutations. *Antimicrob Agents Chemother* 1993; **37**: 457–63.

12 The European *Helicobacter pylori* Study Group. Current European concepts in the management of *Helicobacter pylori* infection. The Maastricht Consensus Report. *Gut* 1997; **41**: 8–13.

13 Torres J, Camorlinga-Ponce M Perez-Perez G *et al*. Increasing multidrug resistance in *Helicobacter pylori* strains isolated from children and adults in Mexico. *J Clin Microbiol*. 2001: **39** (7): 2677–80.

14 Dent JC, McNulty CAM. Evaluation of a new selective medium for *Campylobacter pylori. Eur J Clin Microbiol Inf Dis* 1988; **7**: 555–8.

15 Baeur AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. *Am J Clin Pathol* 1966; **45**: 493–6.

16 Henriksen JH, Broson O, Schogen R, Thoresen T. Risks related to lack of standardization of tests to detect *in vitro* metronidazole resistance in *Helicobacter pylori. Eur J Clin Microbiol Infect. Dis* 1996; **15**: 484–8.

17 *Laboratory methods for the rational use of antibiotics.* AB Biodisk, Pyramidgen 7. S–17136, Solna, Sweden.

18 National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial susceptibility testing*. Ninth informational supplement. Wayne, PA: NCCLS, 1999, Document M100-S9 (19).

19 Marais A, Monteiro L, Ochiallini M, Pina M, Lamouliatte H, Megraud 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 Dore MP, Piana A, Carta M, Atzei A, Are BM, Mura I. Amoxycillin resistance is one reason for failure of amoxycillin omeprazole treatment of *Helicobacter pylori* infection. *Aliment Pharmacol Ther* 1998; **112**: 635–9.

21 Dore MP, Leaudro G, Realdi G, Sepulveda AR, Graham DY. Effect of pretreatment antibiotic resistance to metronidazole and clarithromycin on outcome of *Helicobater pylori* therapy; a meta-analytical approach. *Dig Dis Sci* 2000; **45**: 68–76.

22 Perri F, Festa V, Clemente R, Quitadamo M, Andriulli A. Rifabutin-based 'rescue therapy' for *Helicobacter pylori*-infected patients after failure of standard regimens. *Aliment Pharmacol Ther* 2000; **14**: 311–6.

23 Ani AE, Malu AO, Onah JA, Quieroz DM, Kirschner G, Rocha GA. Antimicrobial susceptibility test of *Helicobacter pylori* isolated from Jos, Nigeria. *Trans R Soc Trop Med Hyg* 1999; **93**: 659–61.

24 Butzler JP, Glupczynski Y, Goosseus H. *Campylobacter* and *Helicobacter* infection. *Curr Opin Infect Dis* 1992; **5**: 80–7.

25 de Boer W, Driessen W, Jansz A, Tytgat G. Effect of acid suppression on efficacy of treatment for *Helicobacter pylori* infection. *Lancet* 1995; **345**: 817–20.

26 Odugbemi T. The use and abuse of antibiotics. *Niger Med Pract* 1981; **1**: 4–8.

27 Rozynek E, Dzierzzanowsha D, Cehiskacedro D, Socha J. Primary resistance of *H. pylori* to macrolides in children. *J Clin Microbiol Infect* 1998; **3**: 231.