Journal of Medical Microbiology (2009), **58**, 930–935 DOI 10.1099/jmm.0.009993-0

Prevalence of multidrug-resistant *Helicobacter pylori* in Bulgaria

Lyudmila Boyanova

Chair of Microbiology, Medical University of Sofia, Sofia, Bulgaria

Correspondence
Lyudmila Boyanova
l.boyanova@hotmail.com

The aim of this study was to evaluate the presence and prevalence of multidrug antibacterial resistance in *Helicobacter pylori* in Bulgaria from 2005 to 2008. The resistance in 828 untreated adults, 124 treated adults and 105 untreated children was, respectively, 26.5, 50.8 and 16.2 % for metronidazole; 18.4, 45.2 and 19 % for clarithromycin; 1, 2.4 and 0 % for amoxicillin; 4.4, 10.6 and 1.9 % for tetracycline; and 9, 14.5 and 5.8 % for ciprofloxacin. Triple resistance to the evaluated agents was uncommon and was detected in 1 % of the untreated children, 3.5 % of the untreated adults and 13.6 % of the treated adults. Five *H. pylori* strains were resistant to amoxicillin, metronidazole and clarithromycin, two of them exhibiting quadruple resistance. Resistance to four of the five antibacterials tested was found in 0.7 % of the untreated and 1.8 % of the treated adults. The overall level of multidrug resistance in the treated adults (15.4 %) was higher than that in the untreated adults (4.2 %, $P=0.0001$) and the untreated children (1 %, $P=0.0001$). The presence of multidrug *H. pylori* resistance in Bulgaria could be associated with many factors, among them the slightly increasing national use of macrolides, lincosamides and streptogramins and of quinolones since 2000, the significant increase in primary *H. pylori* clarithromycin resistance, the high tetracycline use between 1994 and 1999, and, in individual cases, the use of azithromycin-based regimens or reuse of nitroimidazoles. In conclusion, for the first time in a European country during the last 5 years, *H. pylori* strains harbouring a worrying quadruple antibacterial resistance were found in treated as well as in untreated patients. *H. pylori* susceptibility patterns have a tendency to become unpredictable and should be monitored constantly at both national and global levels.

Received 3 February 2009
Accepted 13 March 2009

INTRODUCTION

One important reason for the failure of *Helicobacter pylori* eradication is antibacterial resistance (Morgner *et al.*, 2006). This resistance is most often due to point mutations and can result from inappropriate or frequent antibiotic use (Megraud & Lehours, 2007). The genes responsible for the mutations are the 23S rRNA gene for the macrolides, *rdxA* and *frxA* for metronidazole, *gyrA* for the quinolones, *rpoB* for rifampin, *pbp1* for amoxicillin and the 16S rRNA gene for tetracycline (Megraud & Lehours, 2007). In addition, some non-specific proteins, such as HP1092 and the *hefC* gene product, have been associated with *H. pylori* multidrug resistance (Kutschke & de Jonge, 2005; Saidijam *et al.*, 2006).

In adults, the primary *H. pylori* resistance rates to clarithromycin vary from 0 to 25 % (Megraud & Lehours, 2007). Macrolide resistance is sometimes higher in children than in adults because children are treated with macrolides for respiratory infections more often than adults (Koletzko *et al.*, 2006). Primary resistance rates to metronidazole have been 20–40 % in the USA and Europe, but in developing countries, the rates have been higher (from 50 to >80 %), whilst conversely, in Japan, the rates have been low (1.1–12 %) (Kobayashi *et al.*, 2007; Megraud & Lehours, 2007). Primary *H. pylori* resistance to amoxicillin is uncommon (often 0–2 %) and has been detected in only a few countries; similarly, tetracycline resistance is low except for several countries such as South Korea and Taiwan (Hu *et al.*, 2007). Conversely, because of the increasing use of fluoroquinolones in many countries, quinolone resistance in *H. pylori* has increased and has reached >20 % in adult patients in Japan and Portugal (Megraud & Lehours, 2007; Miyachi *et al.*, 2006). Post-treatment resistance to gatifloxacin has been found to be 47.9 % in Japan (Nishizawa *et al.*, 2006). The double-drug *H. pylori* resistance rate has usually been <10 % in Europe (Koletzko *et al.*, 2006; Megraud & Lehours, 2007), whilst triple resistance to amoxicillin, metronidazole and clarithromycin in *H. pylori* has been only occasional (Torres *et al.*, 2001).

Abbreviations: BST, breakpoint susceptibility testing; DID, defined daily doses per 1000 inhabitants per day; GORD, gastro-oesophageal reflux disease; PPI, proton pump inhibitor.

The aim of the present study was to evaluate the presence and prevalence of multidrug-resistant *H. pylori* in Bulgaria between 2005 and 2008.

METHODS

Patients and specimens. A total of 1057 consecutive *H. pylori* strains, isolated in 2005–2008, were evaluated, comprising strains from untreated adults aged 18–87 years (mean age 43.5 years, 828 cases), untreated children aged 3–17 years (mean age 12.0 years, 105 cases) and treated adults aged 18–75 years (mean age 46.5 years, 124 cases). The untreated children comprised 46 boys and 59 girls, the untreated adults 471 men and 357 women, and the treated adults 72 men and 52 women. The untreated children had chronic gastritis (86 cases), duodenal ulcer (nine cases), gastric ulcer (one case), gastro-oesophageal reflux disease (GORD; five cases) and other diseases (four cases). The untreated and treated adults had chronic gastritis (428 and 60 cases, respectively), duodenal ulcer (200 and 28 cases), gastric ulcer (58 and 12 cases), gastric cancer (six and one cases), GORD (125 and 16 cases) and other diseases, such as gastric polyp and hiatal hernia (11 and seven cases). Informed written consent was obtained from all adults and the parents of all children. The isolation and identification of strains were performed as described previously (Boyanova *et al.*, 2008). Specimens from the treated patients were taken at least 1 month after the end of the *H. pylori* treatment. The most common eradication regimens involved: (i) a proton pump inhibitor (PPI; omeprazole or esomeprazole) + amoxicillin + clarithromycin (38 cases); (ii) PPI + amoxicillin + metronidazole (22 cases); (iii) PPI + amoxicillin + azithromycin (three cases); (iv) PPI + clarithromycin + metronidazole (one case); (v) metronidazole + tetracycline + bismuth compounds + PPI (four cases); (vi) PPI + amoxicillin + clarithromycin + bismuth compounds (one case); and (vii) more than one regimen (five cases). No data were available for the previous treatment of the remaining 50 patients.

Microbiology. The breakpoint susceptibility testing (BST) method is a simplified agar dilution method, using one to four consecutive concentrations of the antibacterial agent. In our previous study, the category agreement between the BST and the Etest or agar dilution method results was found to be high (93.3–100 %) (Boyanova *et al.*, 2008). In the present study, BST was used for susceptibility testing of *H. pylori* as described previously (Boyanova *et al.*, 2008). Briefly, *H. pylori* suspensions were inoculated onto Mueller–Hinton blood agar plates (National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria) containing one of the following drug concentrations: 8, 16 and 32 μg metronidazole ml^{-1}; 0.25, 0.5, 1 and 2 μg clarithromycin ml^{-1}; 0.5, 1 and 2 μg amoxicillin ml^{-1}; 4 μg tetracycline ml^{-1}; and 1 μg ciprofloxacin ml^{-1}. Susceptibility testing for ciprofloxacin was carried out as a marker for strain susceptibility to newer quinolones such as levofloxacin (Megraud & Lehours, 2007). The plates were incubated microaerophilically (Campy Pak; BBL) at 35 °C for 2–3 days. Non-selective Mueller–Hinton blood agar plates were used as a control of strain viability.

The susceptibility patterns of 15 strains with multidrug resistance (eight randomly selected strains with triple resistance and all seven strains with quadruple resistance) were also evaluated by the agar dilution method for metronidazole and Etest (AB Biodisk and Oxoid) for the other agents. Bacterial suspensions (density of 2–3 McFarland standards) were prepared in Mueller–Hinton broth and inoculated onto Mueller–Hinton agar with 5 % sheep blood. Etest strips were placed on the plates (one strip per 90 mm diameter plate) and the plates were incubated at 35 °C for 48–72 h in microaerophilic conditions (as above). The results were read according to the supplier's recommendations.

The breakpoints for resistance were >8 μg metronidazole ml^{-1}, ⩾1 μg clarithromycin ml^{-1}, >0.5 μg amoxicillin ml^{-1}, >4 μg tetracycline ml^{-1} and >1 μg ciprofloxacin ml^{-1} (Megraud *et al.*, 1999; NCCLS, 2000; Megraud & Lehours, 2007, Glocker *et al.*, 2007). Secondary resistance was defined as resistance acquired during treatment by a strain that was susceptible to the agent before treatment. The control strains used for the BST and agar dilution method were two laboratory *H. pylori* isolates with known MICs, as well as *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Bacteroides fragilis* ATCC 25285 (with an appropriate anaerobic incubation).

Statistical analysis. Differences between the groups were assessed with a χ^2 test or Fisher's exact test, as appropriate. P values <0.05 were considered significant.

RESULTS AND DISCUSSION

In the present study, *H. pylori* resistance rates in untreated adults and children were found to be: metronidazole 26.5 % (218/822 patients) and 16.2 % (17/105), respectively; clarithromycin 18.4 % (152/828) and 19.0 % (20/105); amoxicillin 1.0 % (8/825) and 0.0 % (0/105); tetracycline 4.4 % (33/744) and 1.9 % (2/103); ciprofloxacin 9.0 % (71/787) and 5.8 % (6/103); metronidazole + clarithromycin 8.0 % (66/822) and 6.7 % (7/105); and amoxicillin + metronidazole + clarithromycin 0.2 % (2/822) and 0 % (0/105). Primary resistance to metronidazole was significantly more common in untreated adults (26.5 %, 218/822 patients) than in untreated children (16.2 %, 17/105, P=0.022), whereas the differences in the resistance rates to the other agents were not significant (P ⩾0.278).

The primary resistance rates of *H. pylori* were in the range of those frequently reported in Europe with slightly higher amoxicillin and tetracycline resistance rates and a lower metronidazole resistance rate in children. The primary resistance rate to clarithromycin was similar to that found in eastern and southern Europe (usually about 18 %) (Megraud & Lehours, 2007).

The reported resistance of *H. pylori* in treated children and adults (35–68 % to metronidazole, 17–63 % to clarithromycin and 15–73 % to metronidazole + clarithromycin) can hinder the success of eradication (Chisholm *et al.*, 2007; Gosciniak *et al.*, 2004; Kalach *et al.*, 2007; Koletzko *et al.*, 2006; Toracchio & Marzio, 2003; Tüzün *et al.*, 2008). With the increasing number of prescriptions for *H. pylori* eradication and the involvement of new treatment regimens, post-treatment resistance to amoxicillin, tetracycline and quinolones has also been reported (Hsu *et al.*, 2008; Koletzko *et al.*, 2006; Nishizawa *et al.*, 2006). Hsu *et al.* (2008) detected *H. pylori* resistance to amoxicillin and levofloxacin in 17 and 22 % of patients, respectively, after treatment with rabeprazole, bismuth compounds, amoxicillin and levofloxacin.

In the present study, *H. pylori* resistance rates in treated adults were: metronidazole 50.8 % (63/124 patients), clarithromycin 45.2 % (56/124), amoxicillin 2.4 % (3/124), tetracycline 10.6 % (13/123), ciprofloxacin 14.5 %

(16/110), metronidazole + clarithromycin 28.2 % (35/124) and amoxicillin + metronidazole + clarithromycin 2.4 % (3/124). The rates of secondary resistance to metronidazole (88.8 %, 8/9 strains) and clarithromycin (57.1 %, 4/7) were high, unlike those to amoxicillin (0 %, 0/16).

Multidrug resistance of *H. pylori* is occasional and found in individual countries or regions, for example in Sardinia, Mexico and Taiwan (Hu *et al.*, 2007; Kwon *et al.*, 2003). Resistance to amoxicillin, metronidazole and clarithromycin was detected in 6.8 % of 44 Chinese children (Chen *et al.*, 2004), as well as in 4 % of the evaluated children and in 10.4 % of the evaluated adults in Mexico (Torres *et al.*, 2001). In the present study, five (0.5 %) *H. pylori* strains of the 1051 strains tested for susceptibility to amoxicillin, metronidazole and clarithromycin were resistant to these three agents, with two of the strains exhibiting quadruple resistance.

Within the strains tested for susceptibility to all five antibacterial agents, the total multidrug resistance rate in treated adults (15.4 %, 17/110 patients) was higher than that in untreated adults (4.2 %, 31/744, P=0.0001) and untreated children (1 %, 1/103, P=0.0001). The triple and quadruple resistance rates were 1 % (1/103 patients) and 0 % (0/103) for the untreated children, 3.5 % (26/744) and 0.7 % (5/744) for the untreated adults, and 13.6 % (15/110) and 1.8 % (2/110), respectively, for the treated adults. It is of note that triple resistance to metronidazole, clarithromycin and tetracycline was found in an untreated 13-year-old girl with chronic gastritis.

It is known that the success of eradication of clarithromycin-resistant strains is 40–70 % lower than that of susceptible strains (Megraud, 2004; Peitz *et al.*, 2002). Metronidazole resistance is less important, usually decreasing the success of eradication by 25 % (Bazzoli *et al.*, 1999; Megraud & Lehours, 2007; Peitz *et al.*, 2002). Amoxicillin resistance of *H. pylori* could also be clinically important. Although Kim *et al.* (2006) reported that amoxicillin resistance in *H. pylori* did not influence the success of eradication, other authors (Domingo *et al.*, 2002) have detected decreased eradication success of strains with MICs $\geqslant$0.032 μg amoxicillin ml^{-1}.

At present, the best-validated first-line regimen for *H. pylori* eradication consists of a PPI + clarithromycin + amoxicillin administered for 7–14 days (Malfertheiner *et al.*, 2007). Within the strains tested for susceptibility to all five antibacterial agents in the present study, no significant differences were found between the resistance rate to both amoxicillin and clarithromycin (0 %, 0/103 patients) in the untreated children and those in the untreated (0.3 %, 2/744, P=1.000) and treated adults (3.6 %, 4/110, P=0.122).

Quadruple resistance of *H. pylori* has not been reported in Europe and the USA during the last 5 years, although a study from India has reported this type of resistance in 2.6 % of isolates (Thyagarajan *et al.*, 2003). Within the strains tested for susceptibility to all five antibacterial agents in the present study, quadruple *H. pylori* resistance was detected in 0.7 % (5/744 patients) of the untreated and in 1.8 % (2/110) of the treated adults (Table 1). This quadruple resistance was found in six men and one woman. These patients had chronic gastritis (three cases), duodenal ulcer (three cases) and GORD (one case), and five cases were untreated and two were treated (Table 2).

Table 1. Multidrug resistance in *H. pylori* between 2005 and 2008

Agents	Untreated children			Untreated adults			Treated adults		
	No. of strains	No. resistant*	% Resistant	No. of strains	No. resistant	% Resistant	No. of strains	No. resistant	% Resistant
Metronidazole + clarithromycin + ciprofloxacin	103	0	0	787	13	1.6	110	4	3.6
Metronidazole + clarithromycin + tetracycline	103	1	1.0	744	5	0.7	123	6	4.9
Metronidazole + tetracycline + ciprofloxacin	103	0	0	744	5	0.7	110	3	2.7
Amoxicillin + metronidazole + clarithromycin	105	0	0	822	1	0.1	124	2	1.6
Amoxicillin + metronidazole + ciprofloxacin	103	0	0	787	1	0.1	110	0	0
Clarithromycin + tetracycline + ciprofloxacin	103	0	0	744	1	0.1	110	0	0
Metronidazole + clarithromycin + tetracycline + ciprofloxacin	103	0	0	744	4	0.5	110	1	0.9
Amoxicillin + metronidazole + clarithromycin + tetracycline	103	0	0	744	1	0.1	123	0	0
Amoxicillin + metronidazole + clarithromycin + ciprofloxacin	103	0	0	787	0	0	110	1	0.9
Total multidrug resistance*	103	1	1.0	744	31	4.2	110	17	15.4

*Within the strains tested for susceptibility to all five antibacterial agents.

Table 2. Characteristics of the seven patients with quadruple resistance, with previous resistance patterns for the treated patients

M, Male; F, female; R, resistant; S, sensitive.

No.	Sex	Age (years)	Disease	Treatment	R/S (MIC; μg ml^{-1})				
					Amoxicillin	Metronidazole	Clarithromycin	Tetracycline	Ciprofloxacin
1	M	63	Duodenal ulcer	Untreated	R (1)	R (>32)	R (256)	R (64)	S (⩽0.125)
2	M	46	Chronic gastritis	Untreated	S (0.125)	R (>32)	R (256)	R (64)	R (>32)
3	M	28	Chronic gastritis	Untreated	S (⩽0.125)	R (>32)	R (6)	R (64)	R (4)
4	M	53	Chronic gastritis	Untreated	S (⩽0.125)	R (>32)	R (256)	R (8)	R (2)
5	F	51	Duodenal ulcer	Untreated	S (⩽0.125)	R (32)	R (6)	R (8)	R (4)
6	M	27	Duodenal ulcer	Treated*	S (⩽0.125)	R (>32)	R (0.016/6‡)	S (0.25)	S (⩽0.125)
				Treated§	S (⩽0.125)	R (>32)	R (6)	R (128)	R (>32)
7	M	42	GORD	Untreated	R (1)	R (>32)	S (0.19)	S (⩽0.125)	R (>32)
				Treated‖	R (1)	R (>32)	R (⩾256)	S (⩽0.125)	R (>32)

*Treated with omeprazole, amoxicillin and metronidazole.
‡Resistant subpopulation.
§Treated with omeprazole, amoxicillin, tinidazole, tetracycline and bismuth compound.
‖No data about the treatment regimen.

The prevalence of *H. pylori* strains with multidrug antibiotic resistances did not increase during the study period. Within the strains tested for susceptibility to all five antibacterial agents, the frequencies of *H. pylori* strains with multidrug antibiotic resistances in 2005–2006 and in 2007–2008 were 2.0 (1/50 cases) and 0.0 % (0/53, P=0.485) for the untreated children, 3.7 (16/436) and 5.2 % (16/308, P=0.312) for the untreated adults, and 19.6 (11/56) and 11.1 % (6/54, P=0.216) for the treated adults.

Except for amoxicillin, the MIC_{90} values of the antibacterial agents against all strains with multidrug resistance were very high (>32 μg ml^{-1}) (Table 3). The MICs for the strains with quadruple resistance to clarithromycin (MIC_{50} 256 μg ml^{-1} and MIC_{90} >256 μg ml^{-1}) and tetracycline (MIC_{50} >32 μg ml^{-1}) were higher than those for strains with triple resistance (MIC_{50} 3 μg ml^{-1}, MIC_{90} 64 μg ml^{-1} for clarithromycin and MIC_{50} 0.5 μg ml^{-1} for tetracycline).

H. pylori strains harbouring triple or quadruple resistance could hinder the choice and success of the eradication regimen. According to one study, treatment using triple combinations containing amoxicillin was unsuccessful in a patient with *H. pylori* resistance to amoxicillin, clarithromycin and metronidazole (Han *et al.*, 1999). In Korea, 89.6 % of patients with eradication failure have been found to harbour *H. pylori* strains resistant to two or more antimicrobial agents (Kim, 2006).

Table 3. MICs (μg ml^{-1}) of antibacterial agents against 15 multidrug-resistant *H. pylori* isolates by the agar dilution method (ADM) for metronidazole and Etest for the other agents

Agent (method)	MIC_{50}	MIC_{90}	Range
Clarithromycin (Etest)	6	>256	0.023 to >256
Metronidazole (ADM)	>32	>32	0.25 to >32
Amoxicillin (Etest)	⩽0.125	1	⩽0.125 to 1
Tetracycline (Etest)	8	>32	⩽0.125 to >32
Ciprofloxacin (Etest)	4	>32	⩽0.125 to >32

Of the 48 strains with multidrug resistance to metronidazole and other agents, eight (16.7 %) had MICs of 16 μg metronidazole ml^{-1}. For similar strains, a quadruple therapy with lansoprazole, bismuth subsalicylate, metronidazole (reuse) and tetracycline for 14 days was found to be effective in >70 % of patients (Magaret *et al.*, 2001). Several empirical 'rescue' therapy regimens have been recommended for *H. pylori* eradication after failure of two eradication treatments, for example amoxicillin/PPI at high doses, rifabutin/amoxicillin/PPI or furazolidone/bismuth/tetracycline/PPI (Gisbert & Pajares, 2005). Adding non-antimicrobial agents (e.g. lactobacilli) to the eradication regimens can be beneficial to increase the eradication rate by up to 10 % or to minimize the side effects (Lesbros-Pantoflickova *et al.*, 2007). It is important to retreat unsuccessfully treated patients using a case-by-case approach and to perform a susceptibility-guided retreatment if available (Di Mario *et al.*, 2006).

Within the treated patients with a known previous eradication regimen, multidrug resistance was detected after treatment with PPI + amoxicillin + metronidazole (3/22 cases), PPI + amoxicillin + clarithromycin (2/38), PPI + amoxicillin + azithromycin (1/3), PPI + metronidazole + tetracycline + bismuth compounds(1/4), and in more than one regimen (1/5). The presence of multidrug resistance in *H. pylori* in Bulgaria could be associated with the slightly increasing national use of macrolides, lincosamides and streptogramins [J01F, 1.75 defined daily doses per 1000 inhabitants per day (DID) in 2006] and

quinolones (J01M, 1.79 DID in 2006) since 2000 (European Surveillance of Antimicrobial Consumption data for 2006; http://www.esac.ua.ac.be/). Although its use has been decreasing since 2000 (to 2.42 DID in 2006), tetracycline use was very high (>4.2 DID) from 1994 to 1999 (Markova *et al.*, 2005). Within the European countries in 2006, Bulgaria has been a country of moderate total antibiotic use (Muller *et al.*, 2007). However, it is of note that, in Bulgaria, primary clarithromycin resistance in *H. pylori* has increased significantly from 10 % in 1996–1999 to 17.9 % in 2005–2007 (Boyanova *et al.*, 2008). Overall primary metronidazole resistance was stable during this period. Other reasons for unsuccessful eradication and the appearance of multidrug resistance could be the use of azithromycin-based triple regimens in three cases, including one case with triple resistance of the strain, and the reuse of nitroimidazoles in one case. Anagnostopoulos *et al.* (2003) reported successful eradication after azithromycin-based triple regimens in only 62–71 % of the evaluated patients.

In conclusion, multidrug (triple and quadruple) resistance to the key antibacterial agents for eradication of *H. pylori* infection was generally uncommon but was present in 1 % (1/103 cases) of untreated children, in 4.2 % (31/744) of untreated adults and in a higher proportion (15.4 %, 17/110) of treated adults. Eradication of *H. pylori* strains harbouring multidrug resistance requires susceptibility testing of the isolate and should be determined with caution for individual patients. *H. pylori* susceptibility patterns tend to become unpredictable and should be monitored constantly at both national and global levels.

REFERENCES

Anagnostopoulos, G. K., Kostopoulos, P., Margantinis, G., Tsiakos, S. & Arvanitidis, D. (2003). Omeprazole plus azithromycin and either amoxicillin or tinidazole for eradication of *Helicobacter pylori* infection. *J Clin Gastroenterol* **36**, 325–328.

Bazzoli, F., Berretti, D., De Luca, L., Nicolini, G., Pozzato, P., Fossi, S. & Zagari, M. (1999). What can be learnt from the new data about antibiotic resistance? Are there any practical clinical consequences of *Helicobacter pylori* antibiotic resistance? *Eur J Gastroenterol Hepatol* **11** (Suppl. 2), S39–S42.

Boyanova, L., Gergova, G., Nikolov, R., Davidkov, L., Kamburov, V., Jelev, C. & Mitov, I. (2008). Prevalence and evolution of *Helicobacter pylori* resistance to 6 antibacterial agents over 12 years and correlation between susceptibility testing methods. *Diagn Microbiol Infect Dis* **60**, 409–415.

Chen, J., Chen, F. B., Yu, J. D., Chen, X. J., Li, Z. Y. & Zhang, X. P. (2004). Prevalence of *Helicobacter pylori* resistant to clarithromycin, amoxicillin and metronidazole in children. *Zhonghua Er Ke Za Zhi* **42**, 769–771.

Chisholm, S. A., Teare, E. L., Davies, K. & Owen, R. J. (2007). Surveillance of primary antibiotic resistance of *Helicobacter pylori* at centres in England and Wales over a six-year period (2000–2005). *Euro Surveill* **12**, E3–E4.

Di Mario, F., Cavallaro, L. G. & Scarpignato, C. (2006). 'Rescue' therapies for the management of *Helicobacter pylori* infection. *Dig Dis* **24**, 113–130.

Domingo, D., Alarcón, T., Vega, A. E., García, J. A., Martínez, M. J. & López-Brea, M. (2002). Microbiological factors that influence the eradication of *Helicobacter pylori* in adults and children. *Enferm Infecc Microbiol Clin* **20**, 431–434.

Gisbert, J. P. & Pajares, J. M. (2005). Helicobacter pylori ''rescue'' therapy after failure of two eradication treatments. *Helicobacter* **10**, 363–372.

Glocker, E., Stueger, H. P. & Kist, M. (2007). Quinolone resistance in *Helicobacter pylori* isolates in Germany. *Antimicrob Agents Chemother* **51**, 346–349.

Gosciniak, G., Iwanczak, B., Przondo-Mordarska, A., Grabinska, J. & Iwanczak, F. (2004). High level of resistance to metronidazole and clarithromycin in *Helicobacter pylori* isolated from pediatric patients in Poland (1997–2001). *Folia Microbiol (Praha)* **49**, 133–136.

Han, S. R., Bhakdi, S., Maeurer, M. J., Schneider, T. & Gehring, S. (1999). Stable and unstable amoxicillin resistance in *Helicobacter pylori*: should antibiotic resistance testing be performed prior to eradication therapy? *J Clin Microbiol* **37**, 2740–2741.

Hsu, P. I., Wu, D. C., Chen, A., Peng, N. J., Tseng, H. H., Tsay, F. W., Lo, G. H., Lu, C. Y., Yu, F. J. & Lai, K. H. (2008). Quadruple rescue therapy for *Helicobacter pylori* infection after two treatment failures. *Eur J Clin Invest* **38**, 404–409.

Hu, C. T., Wu, C. C., Lin, C. Y., Cheng, C. C., Su, S. C., Tseng, Y. H. & Lin, N. T. (2007). Resistance rate to antibiotics of *Helicobacter pylori* isolates in eastern Taiwan. *J Gastroenterol Hepatol* **22**, 720–723.

Kalach, N., Serhal, L., Asmar, E., Campeotto, F., Bergeret, M., Dehecq, E., Spyckerelle, C., Charkaluk, M. L., Decoster, A. & other authors (2007). *Helicobacter pylori* primary resistant strains over 11 years in French children. *Diagn Microbiol Infect Dis* **59**, 217–222.

Kim, J. M. (2006). Antibiotic resistance of *Helicobacter pylori* isolated from Korean patients. *Korean J Gastroenterol* **47**, 337–349.

Kim, N., Kim, J. M., Kim, C. H., Park, Y. S., Lee, D. H., Kim, J. S., Jung, H. C. & Song, I. S. (2006). Institutional difference of antibiotic resistance of *Helicobacter pylori* strains in Korea. *J Clin Gastroenterol* **40**, 683–687.

Kobayashi, I., Murakami, K., Kato, M., Kato, S., Azuma, T., Takahashi, S., Uemura, N., Katsuyama, T., Fukuda, Y. & other authors (2007). Changing antimicrobial susceptibility epidemiology of *Helicobacter pylori* strains in Japan between 2002 and 2005. *J Clin Microbiol* **45**, 4006–4010.

Koletzko, S., Richy, F., Bontems, P., Crone, J., Kalach, N., Monteiro, M. L., Gottrand, F., Celinska-Cedro, D., Roma-Giannikou, E. & other authors (2006). Prospective multicentre study on antibiotic resistance of *Helicobacter pylori* strains obtained from children living in Europe. *Gut* **55**, 1711–1716.

Kutschke, A. & de Jonge, B. L. (2005). Compound efflux in *Helicobacter pylori*. *Antimicrob Agents Chemother* **49**, 3009–3010.

Kwon, D. H., Dore, M. P., Kim, J. J., Kato, M., Lee, M., Wu, J. Y. & Graham, D. Y. (2003). High-level β-lactam resistance associated with acquired multidrug resistance in *Helicobacter pylori*. *Antimicrob Agents Chemother* **47**, 2169–2178.

Lesbros-Pantoflickova, D., Corthésy-Theulaz, I. & Blum, A. L. (2007). *Helicobacter pylori* and probiotics. *J Nutr* **137** (Suppl. 2), 812S–818S.

Magaret, N., Burm, M., Faigel, D., Kelly, C., Peterson, W. & Fennerty, M. B. (2001). A randomized trial of lansoprazole, amoxycillin, and clarithromycin versus lansoprazole, bismuth, metronidazole and tetracycline in the retreatment of patients failing initial *Helicobacter pylori* therapy. *Dig Dis* **19**, 174–178.

Malfertheiner, P., Megraud, F., O'Morain, C., Bazzoli, F., El-Omar, E., Graham, D., Hunt, R., Rokkas, T., Vakil, N., Kuipers, E. J. & The European Helicobacter Study Group (EHSG) (2007). Current

concepts in the management of *Helicobacter pylori* infection: the Maastricht III Consensus Report. *Gut* **56**, 772–781.

Markova, B., Proevcska, J., Benisheva, T., Valcheva, J. & Popova, M. (2005). Antibiotic use and microbial resistance: data from the ESCMID projects EARSS and ESAC for Bulgaria. In *Proceedings of the 1st Conference of the Bulgarian Association of Medical Microbiology: Rational Antibiotic Policy*, Sofia, Bulgaria.

Megraud, F. (2004). *H. pylori* antibiotic resistance: prevalence, importance, and advances in testing. *Gut* **53**, 1374–1384.

Megraud, F. & Lehours, P. (2007). *Helicobacter pylori* detection and antimicrobial susceptibility testing. *Clin Microbiol Rev* **20**, 280–322.

Mégraud, F., Lehn, N., Lind, T., Bayerdörffer, E., O'Morain, C., Spiller, R., Unge, P., van Zanten, S. V., Wrangstadh, M. & Burman, C. F. (1999). Antimicrobial susceptibility testing of *Helicobacter pylori* in a large multicenter trial: the MACH 2 study. *Antimicrob Agents Chemother* **43**, 2747–2752.

Miyachi, H., Miki, I., Aoyama, N., Shirasaka, D., Matsumoto, Y., Toyoda, M., Mitani, T., Morita, Y., Tamura, T. & other authors (2006). Primary levofloxacin resistance and *gyrA/B* mutations among *Helicobacter pylori* in Japan. *Helicobacter* **11**, 243–249.

Morgner, A., Labenz, J. & Miehlke, S. (2006). Effective regimens for the treatment of *Helicobacter pylori* infection. *Expert Opin Investig Drugs* **15**, 995–1016.

Muller, A., Coenen, S., Monnet, D. L., Goossens, H. & ESAC Project Group (2007). European Surveillance of Antimicrobial Consumption (ESAC): outpatient antibiotic use in Europe, 1998–2005. *Euro Surveill* **12**, E071011.1.

NCCLS (2000). *Performance Standards for Antimicrobial Susceptibility Testing*. Tenth Informational Supplement. Approved standard M100-S10 (M7). Wayne, PA: National Committee for Clinical Laboratory Standards.

Nishizawa, T., Suzuki, H., Kurabayashi, K., Masaoka, T., Muraoka, H., Mori, M., Iwasaki, E., Kobayashi, I. & Hibi, T. (2006). Gatifloxacin resistance and mutations in *gyrA* after unsuccessful *Helicobacter pylori* eradication in Japan. *Antimicrob Agents Chemother* **50**, 1538–1540.

Peitz, U., Sulliga, M., Wolle, K., Leodolter, A., Von Arnim, U., Kahl, S., Stolte, M., Borsch, G., Labenz, J. & Malfertheiner, P. (2002). High rate of post-therapeutic resistance after failure of macrolide-nitroimidazole triple therapy to cure *Helicobacter pylori* infection: impact of two second-line therapies in a randomized study. *Aliment Pharmacol Ther* **16**, 315–324.

Saidijam, M., Benedetti, G., Ren, Q., Xu, Z., Hoyle, C. J., Palmer, S. L., Ward, A., Bettaney, K. E., Szakonyi, G. & other authors (2006). Microbial drug efflux proteins of the major facilitator superfamily. *Curr Drug Targets* **7**, 793–811.

Thyagarajan, S. P., Ray, P., Das, B. K., Ayyagari, A., Khan, A. A., Dharmalingam, S., Rao, U. A., Rajasambandam, P., Ramathilagam, B. & other authors (2003). Geographical difference in antimicrobial resistance pattern of Helicobacter *pylori* clinical isolates from Indian patients: multicentric study. *J Gastroenterol Hepatol* **18**, 1373–1378.

Toracchio, S. & Marzio, L. (2003). Primary and secondary antibiotic resistance of *Helicobacter pylori* strains isolated in central Italy during the years 1998–2002. *Dig Liver Dis* **35**, 541–545.

Torres, J., Camorlinga-Ponce, M., Pérez-Pérez, G., Madrazo-De la Garza, A., Dehesa, M., González-Valencia, G. & Muñoz, O. (2001). Increasing multidrug resistance in *Helicobacter pylori* strains isolated from children and adults in Mexico. *J Clin Microbiol* **39**, 2677–2680.

Tüzün, Y., Bayan, K., Yilmaz, S., Dursun, M. & Ozekinci, T. (2008). The prevalence of primary and secondary *Helicobacter pylori* resistance to clarithromycin and probable contributing cofactors: data from southeastern Anatolia. *Hepatogastroenterology* **55**, 289–293.