JMM Correspondence

Helicobacter pylori infection in elderly Bulgarian patients

Helicobacter pylori infection causes chronic gastritis that can trigger peptic ulcer disease and gastric malignancy (Marshall, 1994). The serious complications of peptic ulcers (bleeding or perforation) affect many elderly patients worldwide (Pilotto, 2001). Successful eradication of the infection results in ulcer healing (Adamek *et al.*, 1998) and may prevent the progression of intestinal metaplasia in elderly patients (Pilotto & Malfertheiner, 2002); however, clinical interest in *H. pylori* infection in elderly people remains low (Pilotto & Salles, 2002). Furthermore, gastroduodenal diseases are associated with other diseases in many old patients. The aim of this study was to evaluate the prevalence of *H. pylori* infection and primary *H. pylori* resistance to antimicrobial agents in elderly Bulgarian patients with gastroduodenal diseases.

A total of 127 consecutive elderly patients (60–89 years) with gastroduodenal diseases between January 1996 and July 2003 were evaluated. All were admitted because of abdominal complaints, mostly consisting of epigastric pain and dyspepsia. No patient had been treated previously for *H. pylori* infection. The classification of diseases as chronic gastritis, erosive gastritis, duodenal ulcer, stomach ulcer and stomach cancer was based on endoscopic findings, histology and clinical signs (Table 1). Endoscopy was performed with an Olympus GIF P20 (biopsy forceps K19). Two stomach biopsy specimens per patient were taken at 3–5 cm from the pylorus. Specimens were transported in Stuart's transport medium (Merck) for less than 5 h. Susceptibility to antimicrobial agents of 92 *H. pylori* isolates was investigated.

A smear was prepared from one part of the specimens by scraping the biopsy on a slide. The smear was used for a modified Gram stain with carbol fuchsin as the counterstain. For the urease test, another part of the biopsy specimen was placed in urea (10 %) agar medium as described previously (Boyanova *et al.*, 1996), incubated at 37 °C and observed for colour change after 30 min and 3 h. The remaining part of the biopsy specimen was homogenized in 0·1 ml sterile saline with sterile needles and inoculated onto blood agar [Columbia agar base (Oxoid) or Brucella agar base (Becton Dickinson)] containing 10 % defibrinated sheep blood and 1 % Isovitalex (Becton Dickinson), with or without *H. pylori* selective supplement, containing 10 μg vancomycin, 5 μg trimethoprim, 5 μg cefsulodin and 5 μg amphotericin B ml^{-1} [*H. pylori* selective supplement (Dent); Oxoid]. One selective and one non-selective medium plate were used for primary culture of biopsy specimens. Plates were incubated for 12 days in a microaerobic atmosphere. *H. pylori* was identified by Gram staining of suspect colonies, lack of aerobic growth on blood-agar plates and testing for the presence of urease, oxidase and catalase. Specimens were considered as *H. pylori* positive only if culture or two of the other three diagnostic methods gave positive results.

In the screening agar method (SAM), two drops (approx. 60 μl) of *H. pylori* suspensions, prepared in Mueller–Hinton broth (NCIPD) to obtain McFarland turbidity standard 3–4, were inoculated on a quarter of the surface of Mueller–Hinton blood-agar plates (NCIPD) containing 1 % Isovitalex and one of the following drug concentrations: 8, 16 or 32 μg metronidazole ml^{-1}, 0·25, 0·5, 1 or 2 μg clarithromycin or azithromycin ml^{-1}, 0·5, 1 or 2 μg amoxicillin ml^{-1}, 1 μg ciprofloxacin ml^{-1} or 4 μg tetracycline ml^{-1}. Antimicrobial agents were obtained from Sigma (amoxicillin, metronidazole and tetracycline), Abbott Laboratories (clarithromycin), Balkanpharma (azithromycin) and Bayer Pharma (ciprofloxacin). The plates were incubated microaerobically at 37 °C for 3 days. If *H. pylori* growth appeared on the plate, the isolate was considered to be resistant to the corresponding drug. Non-selective medium plates were used as a control of strain viability. Primary resistance rates of 92 strains from elderly patients with gastroduodenal diseases were compared with those of 423 isolates from adults aged 19–59 years.

Minimal inhibitory concentrations (MICs) of clarithromycin were determined for 48 strains from elderly patients and were compared with those of 70 isolates from adults aged 19–59 years. McFarland 3 suspensions were prepared in Mueller–Hinton broth and 0·5 ml volumes were used

Table 1. Patients involved in the present study

Patient group	*n*	*H. pylori*-positive*		
		n	%	95 % CI of percentage
All	127	103	81·1	73·5–88·7
By age				
60–69 years	80	64	80·0	71·2–88·8
70–79 years	43	35	81·4	69·6–93·2
80–89 years	4	4	100·0	72·4–100·0
By condition				
Chronic gastritis	65	51	78·5	68·5–88·5
Erosive gastritis	24	19	79·2	62·6–95·8
Duodenal ulcer	22	22	100·0	92·1–100·0
Stomach ulcer	9	6	66·7	34·1–99·3
Stomach cancer	7	5	71·4	35·2–100·0

*Specimens were considered positive for *H. pylori* only if culture or two of the other three diagnostic methods were positive.

to flood Mueller–Hinton blood-agar plates containing 1 % Isovitalex without antibiotics. E test strips (AB Biodisk) were placed on the plates and they were incubated for 48–72 h under microaerobic conditions. The results were read according to the supplier's recommendations. The breakpoints used to define resistance by SAM and E test were: $>$ 8 μg metronidazole ml^{-1}, $>$ 1 μg clarithromycin and azithromycin 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). Metronidazole resistance was determined from SAM results only.

Differences between patients with susceptible and resistant strains were assessed by χ^2 test with Yates's correction.

Scant data are available concerning both the incidence of *H. pylori* infection and the susceptibility patterns of isolates from elderly patients with dyspeptic complaints (Parsons *et al.*, 2001; Pilotto, 2001; Pilotto & Malfertheiner, 2002; Pilotto & Salles, 2002). In the present study, *H. pylori* infection was found in 103 (81·1 %) elderly patients with gastroduodenal diseases (Table 1). Culture failed to detect *H. pylori* in nine (8·7 %) of the specimens that were positive with other diagnostic tests, probably as a result of the increase in gastric atrophy (Pilotto & Salles, 2002) and intestinal metaplasia or previous treatment for other infections in the older age group. Nevertheless, *H. pylori* infection was found in $>$ 78 % of Bulgarian elderly patients with chronic gastritis and in 90·3 % of those with peptic ulcers, unlike several other studies that have reported *H. pylori* prevalence in elderly patients with peptic ulcers as 50–78 % (Pilotto, 2001; Pilotto & Malfertheiner, 2002; Pilotto & Salles, 2002). There was no statistically significant difference between the prevalence of *H. pylori* infection in elderly patients aged 60–69 years and that in older patients ($P > 0{\cdot}20$).

According to recent studies, better *H. pylori* eradication has been obtained in patients aged over 60 years (except for type 2 diabetic patients) than in younger patients (Broutet *et al.*, 2003; Sargyn *et al.*, 2003). In the present study, the resistance rates to metronidazole, tetracycline and newer macrolides in elderly patients were similar to those in younger adults ($P > 0{\cdot}20$). Amoxicillin resistance was not detected in the elderly but was found in 1 % of strains isolated from younger adults (Table 2). Although ciprofloxacin is not currently used in treatment regimens for *H. pylori* eradication, a slightly higher prevalence of ciprofloxacin resistance was detected in older (8·9 %) than in younger adults (6·1 %) ($P > 0{\cdot}20$). Elderly patients are at higher risk of hospitalization and treatment for respiratory or urinary infections (Pilotto & Salles, 2002). This could explain the rate of ciprofloxacin resistance in older patients.

Table 2. Primary and combined resistance in *H. pylori* isolates from elderly and other adult patients with gastroduodenal disease

Other adults refers to patients aged 19–59 years. Confidence intervals (CI) refer to percentage values.

Agent	Patient group	Strains tested (*n*)	Resistance (%)	95 % CI
Metronidazole	Elderly	92	28·3	19·0–37·6
	Other adults	423	28·8	24·5–33·1
Clarithromycin	Elderly	90	12·2	5·4–19·0
	Other adults	415	11·3	8·3–14·3
Azithromycin	Elderly	59	15·2	6·0–24·4
	Other adults	253	16·2	11·7–20·7
Amoxicillin	Elderly	88	0	0–2·1
	Other adults	361	1·1	0–2·2
Ciprofloxacin	Elderly	45	8·9	0·5–17·3
	Other adults	212	6·1	2·9–9·3
Tetracycline	Elderly	88	4·5	0·2–8·8
	Other adults	419	4·3	2·4–6·2
Metronidazole + clarithromycin	Elderly	86	4·6	0·2–9·1
	Other adults	411	4·3	2·3–6·3

According to several authors, the overall rate of metronidazole resistance has shown a tendency to decrease with age, whereas the prevalence of clarithromycin resistance has not been associated with age (Fraser *et al.*, 1999; Parsons *et al.*, 2001). In the present study, the MIC values of clarithromycin for strains from elderly patients were similar to those from younger adults. Although the prevalence of combined resistance to metronidazole and clarithromycin in the elderly (4·6 %) was similar to that in other adults (4·3 %), fewer (52·6 %) metronidazole-resistant strains from elderly patients had high levels of metronidazole resistance (MIC $>$ 32 μg ml^{-1}) than those from younger adults (78·7 %) ($P < 0{\cdot}001$).

In conclusion, because *H. pylori* infection was detected in most elderly patients with gastroduodenal diseases and the primary resistance rates in the elderly patients were similar to those in younger adults, microbiological diagnostic tests for *H. pylori* infection in this poorly studied group of elderly patients with dyspeptic complaints are necessary and beneficial.

Lyudmila Boyanova,[1] Nikolai Katsarov,[2] Galia Gergova,[1] Rossen Nikolov,[3] Sirigan Derejian,[3] Zoya Spassova,[3] Ivan Mitov[1] and Zacharii Krastev[3]

[1]Department of Microbiology, Medical University of Sofia, Sofia, Bulgaria

[2]Second Surgery Department of Alexander Hospital, Sofia, Bulgaria

[3]Department of Gastroenterology, University Hospital 'St Ivan Rilski', Sofia, Bulgaria

Correspondence: Lyudmila Boyanova (lboyanova@hotmail.com)

Adamek, R. J., Suerbaum, S., Pfaffenbach, B. & Opferkuch, W. (1998). Primary and acquired *Helicobacter pylori* resistance to clarithromycin, metronidazole, and amoxicillin – influence on treatment outcome. *Am J Gastroenterol* **93**, 386–389.

Boyanova, L., Stancheva, I., Todorov, D. & 7 other authors (1996). Comparison of three urease tests for detection of *Helicobacter pylori* in gastric biopsy specimens. *Eur J Gastroenterol Hepatol* **8**, 911–914.

Broutet, N., Tchamgoue, S., Pereira, E., Lamouliatte, H., Salamon, R. & Megraud, F. (2003). Risk factors for failure of *Helicobacter pylori* therapy –results of an individual data analysis of 2751 patients. *Aliment Pharmacol Ther* **17**, 99–109.

Fraser, A. G., Moore, L., Hackett, M. & Hollis, B. (1999). *Helicobacter pylori* treatment and antibiotic susceptibility: results of a five-year audit. *Aust N Z J Med* **29**, 512–516.

Marshall, B. J. (1994). *Helicobacter pylori*. *Am J Gastroenterol* **89** (Suppl.), S116–S128.

Megraud, F., Lehn, N., Lind, T. & 7 other authors (1999). Antimicrobial susceptibility testing of *Helicobacter pylori* in a large multicenter trial: the MACH 2 study. *Antimicrob Agents Chemother* **43**, 2747–2752.

NCCLS (2000). *Performance standards for antimicrobial susceptibility testing*. Tenth informational supplement. Approved standard M100-S10 (M7). Wayne, PA: National Committee for Clinical Laboratory Standards.

Parsons, H. K., Carter, M. J., Sanders, D. S., Winstanley, T. & Lobo, A. J. (2001). *Helicobacter pylori* antimicrobial resistance in the United Kingdom: the effect of age, sex and socio-economic status. *Aliment Pharmacol Ther* **15**, 1473–1478.

Pilotto, A. (2001). *Helicobacter pylori*-associated peptic ulcer disease in older patients: current management strategies. *Drugs Aging* **18**, 487–494.

Pilotto, A. & Malfertheiner, P. (2002). Review article: an approach to *Helicobacter pylori* infection in the elderly. *Aliment Pharmacol Ther* **16**, 683–691.

Pilotto, A. & Salles, N. (2002). *Helicobacter pylori* infection in geriatrics. *Helicobacter* **7** (Suppl. 1), 56–62.

Sargyn, M., Uygur-Bayramicli, O., Sargyn, H., Orbay, E., Yavuzer, D. & Yayla, A. (2003). Type 2 diabetes mellitus affects eradication rate of *Helicobacter pylori*. *World J Gastroenterol* **9**, 1126–1128.