Prevalence of antibiotic resistance of *Helicobacter pylori* isolates in Estonia during 1995–2000 in comparison to the consumption of antibiotics used in treatment regimens

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Objective To find a possible relation between the dynamics of antibiotic resistance of *Helicobacter pylori* isolates and the consumption of antibiotics during the last several years in Estonia.

Methods *Helicobacter pylori* isolates were collected from the gastric mucosa of patients with peptic ulcer (153) and gastritis (68) and isolated on the Columbia Agar Base. From 1995 to 1997 the disk-diffusion method was used for testing of *H. pylori* susceptibility to metronidazole (115 isolates), erythromycin (119 isolates), tetracycline (119 isolates) and amoxicillin (119 isolates). From 1998 to 2000 the susceptibility of *H. pylori* to metronidazole (106 isolates), amoxicillin (30 isolates), clarithromycin (106 isolates) and ciprofloxacin (30 isolates) was assessed by E tests. Data from the Estonian State Agency of Medicines were used to determine the antibiotic consumption rate.

Results Up to the year 2000 all the investigated *H. pylori* isolates were susceptible to ciprofloxacin; the resistance to clarithromycin, tetracycline, amoxicillin and erythromycin was 3%, 1.7%, 0.7% and 2.5%, respectively. Forty-six percent of *H. pylori* isolates were resistant to metronidazole. During 1995–2000 the consumption of amoxicillin, erythromycin and ciprofloxacin increased and the consumption of tetracycline decreased. The increasing consumption of amoxicillin reached a level 5.7 times than that of the consistent use of metronidazole. The resistance to amoxicillin appeared to be very low and resistance to metronidazole was continuously high. The increase of clarithromycin consumption (from 0.002 to 1.119 defined daily doses/1000) during three years was associated with the appearance of the first clarithromycin-resistant isolates in 2000.

Conclusion No relation was observed between the antibiotic consumption rate and the resistance pattern of *H. pylori* to metronidazole, amoxicillin, erythromycin, tetracycline and ciprofloxacin during recent years among the in population.

Keywords *Helicobacter pylori*, antibiotic susceptibility, consumption of antibiotics

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INTRODUCTION

Helicobacter pylori is found colonizing the stomach of patients with chronic gastritis and peptic ulcer disease [1,2]. Eradication of *H. pylori* has a significant effect on the clinical symptoms and cure rate of peptic ulcer [3]. However, resistance of *H. pylori* to antibiotics can be a cause of treatment failures [4].

A significant difference in the resistance to antibiotics, especially to metronidazole and clarithromycin, has been found among countries. The resistance to metronidazole usually ranges from 10 to 90% [5,6]. The clarithromycin-resistance rate in Europe varies from 0 to 15% [5,6]. The prevalence of resistance to erythromycin, tetracycline and

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amoxicillin is universally very low in Europe [5,7], but cross-mutations conferring resistance to macrolides may exist [8,9]. Data concerning resistance of *H. pylori* to fluoroquinolones are scarce.

There is general agreement that increasing antimicrobial resistance is related to the selective pressure exerted by the use of antibiotics [10]. A higher rate of metronidazole resistance of *H. pylori* has been associated with the extensive use of this inexpensive antibiotic for the treatment of parasitic, genital and dental infections [11]. On the other hand, clarithromycin resistance mainly depends on the intensity of administration of the newly introduced medicine in different countries. In those countries where clarithromycin is widely used for the treatment of respiratory tract infections, the resistance of *H. pylori* isolates is higher than in those where macrolides are rarely used [5,7,11]. In Estonia the population was not treated with clarithromycin before 1997, when clarithromycin was officially registered. This may offer a good opportunity to estimate the development of *H. pylori* resistance.

In other studies, metronidazole- and amoxicillin-resistance rates have remained stable, but clarithromycin-resistant isolates have increased in patients treated with these antibiotics [12]. No data comparing the resistance of *H. pylori* to different antimicrobial agents with the dynamics of antibiotic consumption in the population are available.

The first objective of our study was to assess the prevalence of metronidazole-, clarithromycin-, erythromycin-, tetracycline-, amoxicillin- and ciprofloxacin-resistant isolates of *H. pylori* in Estonia from 1995 to 2000. The second goal was to find the possible relation between the susceptibility pattern of *H. pylori* and the rate of antibiotic use during different years.

METHODS

A total of 221 *H. pylori* isolates were obtained for estimating an antibiotic susceptibility pattern over a period of 6 years (from 25 April 1995 to 18 October 2001) from *H. pylori*-positive adult outpatients (130 men and 91 women), all referred to the Tartu University Outpatients Clinic for upper gastrointestinal endoscopies. None of the patients had a previous history of *H. pylori* infection.

Upper endoscopies were performed by the gastroscope Olympus-GIF 21. Biopsies were obtained with medium-size forceps. Gastric ulcer was diagnosed if the ulcer was located at the angulus or above it. Duodenal ulcer was diagnosed if the ulcer was found in the praepyloric, pyloric, or duodenal bulb area. The resulting endoscopic diagnoses were the following: 153 cases of peptic ulcer (duodenal or gastric ulcer) and 68 cases of normal findings where gastritis was diagnosed histologically according to the Sydney classification [13].

The biopsy samples from the antrum and the corpus were placed into the Stuart Transport Medium and taken to the laboratory within 2 h.

Data from the Estonian State Agency of Medicines were used to assess the antibiotic consumption rate in the population [as defined daily doses (DDD)/1000/annually]. To estimate the differences in dynamics of antibiotic consumption, the data were divided into two periods (from 1995 to 1997 and from 1998 to 2000).

H. pylori was isolated on the Columbia Agar Base supplemented with 7% horse blood and 1% Isovitalex. The plates were incubated for 3–4 days at 37 °C under microaerophilic conditions (CampyGen Oxoid) and Gram staining, oxidase, catalase and urease reactions were used for *H. pylori* identification.

From each patient, one *H. pylori* isolate was tested for susceptibility to metronidazole, amoxicillin, tetracycline, erythromycin, clarithromycin and ciprofloxacin. A suspension was prepared in Brucella broth (2 mL) equivalent to the McFarland turbidity standard 3–4. From this suspension, 100 μL was transferred onto the surface of the Columbia Agar Base with 7% horse blood and 1% Isovitalex and streaked with a cotton swab. Antibiotic disks (BBL, Cockeysville, MD, USA) or strips (the E test; AB Biodisk, Salna, Stockholm, Sweden) were applied onto the surface of inoculated and dried agar plates. For testing antibiotic susceptibility, the plates were incubated in a microaerophilic milieu at 37 °C for 3–4 days before examination [6,14].

From 1995 to 1997, the disk-diffusion method was used for *H. pylori* susceptibility testing to metronidazole (115 isolates), erythromycin, tetracycline and amoxicillin (119 isolates for each). The following inhibition zones were regarded as indicating antibiotic sensitivity of the *H. pylori* isolates: >15 mm for metronidazole (5 μg), ≥ 23 mm for erythromycin (15 μg), ≥ 19 mm for tetracycline (30 μg) and ≥ 17 mm for amoxicillin (30 μg) [7,15].

From 1998 to 2000 the susceptibility of *H. pylori* to metronidazole (106 isolates), amoxicillin (30 isolates), clarithromycin (106 isolates) and ciprofloxacin (30 isolates) was estimated by E tests. Antibiotic breakpoints employed for the E test were $\leq$8.0 mg/L for metronidazole, $\leq$2.0 mg/L for clarithromycin and $\leq$8.0 mg/L for amoxicillin [4,7].

The random 30 *H. pylori* isolates from 30 patients (10 with gastritis and 20 with peptic ulcer) were tested for metronidazole susceptibility by the disk-diffusion method and the E test, comparatively.

H. pylori strain ATCC 43504 was used as a reference strain for quality control of antibiotic susceptibility testing.

Data were analysed using a Jandel SigmaStat 2.0 program, using χ^2 or Fisher Exact Tests.

RESULTS AND DISCUSSION

The consumption rates of some antibiotics in Estonia during 1995–2000, divided into two 3-year periods are presented in Table 1. No change was found in the consumption of metronidazole. The consumption increased 2.4-fold for ciprofloxacin, 2.5-fold for amoxicillin, 1.3-fold for erythromycin and decreased twofold for tetracycline. The use of clarithromycin, a relatively new medicine in Estonia (available since 1997) increased from 0 to 1.19 (DDD/1000). Amoxicillin was the most frequently used antibiotic in 2000. The use of amoxicillin was 5.7-fold higher than that of metronidazole.

Metronidazole-sensitive and -resistant *H. pylori* isolates were recovered in similar proportions: 120 *H. pylori* isolates were metronidazole-sensitive (69 by disk diffusion and 51 by the E test) and 101 were metronidazole-resistant (46 isolates by disk diffusion and 55 by the E test). The resistance to metronidazole of *H. pylori* was 46% (40% by the disk-diffusion method and 52% by the E test). The differences between both methods were not statistically significant ($P > 0.05$).

There were no differences in metronidazole susceptibility of isolates collected from patients with peptic ulcer as compared to those who had only gastritis (data not shown, $P > 0.05$).

From 106 investigated *H. pylori* isolates 103 were sensitive to clarithromycin [minimum inhibitory concentration (MIC) ranges from $<$0.016 to 2 mg/L) and only three were resistant (MIC ranges from 6 to $>$256 mg/L), the resistance rate being 3%.

Among 119 isolates of *H. pylori* the resistance to tetracycline (two isolates; 1.7%) and erythromycin (three isolates; 2.5%) was low. The resistance to amoxicillin (119 isolates tested by disk-diffusion method and 30 by the E test) remained at 0.7% (one isolate). The one isolate, resistant to erythromycin was simultaneously resistant to tetracycline and amoxicillin and the other isolate was resistant to both erythromycin and tetracycline. All 30 *H. pylori* isolates investigated were susceptible to ciprofloxacin.

In 26 isolates (13 resistant and 13 sensitive), equal values were obtained with both methods, four isolates showed a different susceptibility pattern and the discrepancy between both tests was only 13.3%. In one case one isolate was sensitive according to the E test and resistant according to the disk-diffusion method. In three cases the isolates resistant according to the E test were sensitive to metronidazole according to the disk-diffusion method.

The results of susceptibility tests performed in different years are depicted in Table 2. No statistically significant differences were found between the resistance rates of *H. pylori* for the two different 3-year periods or by utilizing the different susceptibility methods. The use of metronidazole and *H.*

Table 1 The differences in the consumption of some antibiotics (DDD/1000[a]) from 1995 to 1997 and from 1998 to 2000 in Estonia

Antibiotic	DDD/1000[a]		Consumption dynamics
	1995–1997	1998–2000	
Metronidazole	0.96 (0.83–1.29)	0.85 (0.80–0.86)	Decrease 1.1-fold
Amoxicillin	1.38 (0.74–2.3)	3.49 (2.80–4.90)	Increase 2.53-fold
Erythromycin	0.98 (0.94–1.03)	1.29 (1.50–1.44)	Increase 1.32-fold
Tetracycline	1.14 (1.10–0.76)	0.42 (0.57–0.35)	Decrease 2.7-fold
Ciprofloxacin	0.13 (0.10–0.16)	0.31 (0.27–0.3)	Increase 2.38-fold
Clarithromycin	0.002[b]	1.11 (0.05–1.19)	Increase from 0 to 1.19

[a]DDD/1000, defined daily doses per 1000 annually; [b]officially registered in 1997.

Table 2 Resistance of *Helicobacter pylori* isolates to antibiotics in Estonia during the last 6 years from (from 25 April 1995 to 31 December 1997 and from 1 January 1998 to 18 October 2000)

	1995–1997		1998–2000	
Antibiotic	***n***	**Res. (%)**	***n***	**Res. (%)**
Metronidazole[a,b]	115	40.0	106	52.0
Amoxicillin[a,b]	99	1.0	50	0
Erythromycin[a]	99	3.0	20	0
Tetracycline[a]	99	2.0	20	0
Ciprofloxacin[b]	0	nd[c]	30	0
Clarithromycin[b]	16	0	90	3.33[d]

n, number of isolates; Res. (%), percent resistance.
[a]Kirby–Bauer disk-diffusion method; [b]the E test; [c]not done; [d]all the clarithromycin-resistant isolates were collected in 2000.

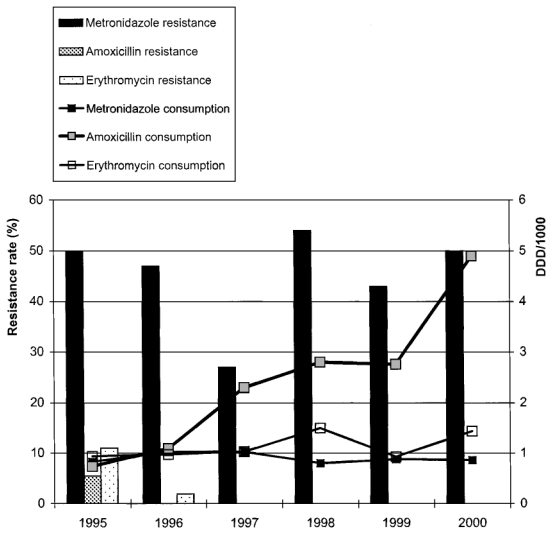

Figure 1 The resistance to antibiotics (%) of *Helicobacter pylori* in comparison with the use rate (DDD/1000).

pylori-resistance patterns was quite stable, as no statistically significant differences ($P > 0.05$) were found (Figure 1). Even though the consumption of amoxicillin increased 2.5-fold, the resistance to this antibiotic remained very low. Nor could the comparatively low consumption of metronidazole be associated with the highest (46%) resistance to metronidazole, discovered by different susceptibility testing methods in the microaerophilic environment. In comparison with the use of metronidazole and amoxicillin, the latter was much higher. However, the resistance to metronidazole was continuously high and the resistance to amoxicillin was very low.

The goal of the study was to estimate the susceptibility of Estonian *H. pylori* isolates to different antibiotics used in the treatment of *H. pylori* infections. The changes in the consumption rate of some antimicrobial agents over a 6-year period were not followed by an increase in antibiotic resistance of *H. pylori* to these drugs. This was not the anticipated result; we expected that the increase or decrease of antibiotic consumption would influence the microbial resistance pattern generally. The extensive use of antibiotics usually leads to an increase of resistance against these drugs. The relation between the use of erythromycin and the resistance of *Streptococcus pyogenes* to this antibiotic has already been verified [16].

Our particular interests were metronidazole, as a medicine, used world-wide for a long period, the macrolides, belonging to the treatment regimens of a wide spectrum of infections for approximately 50 years, and the extensively consumed beta-lactame antibiotics (amoxicillin). The resistance rate of *H. pylori* to these antibiotics shows wide variation in different countries [7,11].

Thanks to the database of the Estonian State Agency of Medicines, it was possible to acquire 6-year comparative data for the total consumption of the antibiotics most frequently used in the treatment regimens for *H. pylori* infection. The dynamics of consumption of these antibiotics was different: the use of metronidazole and erythromycin was quite stable, but the use of clarithromycin increased from zero to 0.19 DDD/1000, use of amoxicillin increased 2.4-fold, use of ciprofloxacin increased 2.5-fold and tetracycline use decreased 2.7-fold. Clarithromycin resistance was not detected among *H. pylori* isolates in the Estonian population from 1997 to 1999, and clarithromycin seemed to be an excellent component of the triple therapy used in the eradication of *H. pylori* up to 2000 when the first resistant isolates

appeared. In comparing the use of metronidazole and amoxicillin, the latter was used at a rate 5.7 times higher. However, the resistance to metronidazole was continuously high and the resistance to amoxicillin remained very low. At the same time, the prevalence of resistance to amoxicillin, erythromycin, tetracycline and ciprofloxacin was very low or completely absent.

Likewise, there is a discrepancy between the resistance and consumption of metronidazole. Although the consumption of metronidazole was lower than that of amoxicillin or ciproflocacin, the resistance pattern was constantly high for 6 consecutive years. In the present study in the year 2000, metronidazole resistance was 50% (generally 46%). The Norwegian metronidazole-resistance rate was similar to that established by our data [17] although the consumption was nearly 180 times lower (DDD/1000 was 0.0056). It is possible that at continuously stable and quite low (1.0 DDD/1000) rate of consumption of metronidazole, the high resistance pattern (46%) of *H. pylori* can be largely attributed to the methodical problems of sensitivity testing in microaerophilic vs. anaerobic milieu [18,19].

At the beginning of our study, we had to use the disk-diffusion method only; later E tests were applied. The susceptibility pattern of *H. pylori*, assessed by the Kirby–Bauer disk-diffusion method, and the E test showed only 13.3% discordance: three isolates, resistant according to the E test were sensitive to metronidazole according to the disk-diffusion method. The reason for this phenomenon may be that the gradient profile around the disk changes over time and a large inhibition zone may in some cases reflect slower growth [20]. These results confirm that the E test is more reliable for studying the resistance of *H. pylori* than the disk-diffusion method in the case of single isolates. In contrast, if we compared the average resistance to metronidazole of numerous isolates, as assessed either by the disk-diffusion method or by the E tests in different years, no statistically significant changes were found. So the results of the disk-diffusion tests are acceptable to estimate *H. pylori* resistance in the population generally.

We did not find any differences in metronidazole susceptibility of the *H. pylori* strains isolated from patients with gastritis compared to those from patients with peptic ulcer disease. Our data, which show no significant correlation between the patients' diagnoses and the resistance patternsapos; are in agreement with the results obtained by other authors, showing that the patients with gastritis and peptic ulcer can be colonized both with resistant and sensitive *H. pylori* isolates [4].

To summarize, we did not observe a relation between the changes in antibiotic consumption (DDD/1000) rate and the resistance pattern of *H. pylori* to amoxicillin, erythromycin, tetracycline and ciprofloxacin during a 6-year follow-up period. The first clarithromycin-resistant isolates appeared in 2000. Therefore, the consumption rate of antibiotics in the Estonian population was not directly associated with the antibiotic-resistance profile of *H. pylori* during the past 6 years.

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