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Antimicrobial Susceptibility Studies

Primary *Helicobacter pylori* resistance in elderly patients over 20 years: A Bulgarian study

Lyudmila Boyanova [a,*], Galina Gergova [a], Rumyana Markovska [a], Nayden Kandilarov [b], Lyubomir Davidkov [c], Zoya Spassova [d], Ivan Mitov [a]

[a] Department of Medical Microbiology, Medical University of Sofia, Zdrave street 2, 1431 Sofia, Bulgaria
[b] Department of General and Hepatobiliary Pancreatic Surgery, Medical University of Sofia, Sofia, Bulgaria
[c] Department of Gastroenterology, University Hospital St. Ekaterina, Medical University of Sofia, Sofia, Bulgaria
[d] Department of Gastroenterology, University Hospital St. Ivan Rilski, Medical University of Sofia, Sofia, Bulgaria

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ABSTRACT

We evaluated the antibiotic susceptibility of 233 *Helicobacter pylori* strains isolated in the period 2011–2016, involving 62 strains from elderly patients aged 66–93 years and 171 strains from younger adults. To assess resistance evolution, primary resistance rates in 92 strains from as many patients aged ≥60 years in 1996–2003 were compared with those in 85 strains from infected patients in the same age group in 2011–2016. In the patients aged >65 years evaluated during the last 6 years, amoxicillin resistance according to EUCAST and prior breakpoints was 1.6 and 0%, respectively. Resistance rates were the same by both breakpoint systems to metronidazole (35.5%), clarithromycin (22.6%), tetracycline (1.6%) and levofloxacin (32.3%). In 2011–2016, there were no significant differences between resistance rates in the subjects aged >65 years and the younger adults. Notably, during the last 6 years, double/triple resistance was found in 21.0% of the subjects aged >65 years. Moreover, the prevalence of quinolone primary resistance (30.0%) was significantly (3.4-fold) higher than that (8.9%) observed in 1996–2003. Briefly, the presence of both combined resistance and a strikingly high primary levofloxacin resistance in the elderly implies a cautious antibiotic choice for *H. pylori* eradication. *In vitro* susceptibility testing of the strains is highly important in this age group. The results can be linked to more frequent comorbidities and co-infection treatment in older compared with younger patients and, additionally, to the national antibiotic consumption. The high prevalence of quinolone resistance in the elderly patients is an alarming finding.

1. Introduction

Helicobacter pylori is recognized as a class I carcinogen, playing a major oncogenic role in the development of gastric cancer, the third leading cause of cancer death worldwide, and is also linked to mucosa-associated lymphoid tissue (MALT) lymphoma as well as to chronic gastritis and peptic ulcers (Conteduca et al., 2013; O'Connor et al., 2017). Because of a birth-cohort phenomenon, *H. pylori* infection prevalence increases with increasing age, reaching levels of >70% in elderly patients with gastroduodenal diseases (Pilotto and Franceschi, 2014).

In our recent study (Yordanov et al., 2016) on 69 blood donors aged 51–69 years, *H. pylori* seroprevalence was high (81.2%) and cytotoxin-associated gene A (CagA) IgG prevalence was 58.0%. CagA seropositivity is associated with a significantly increased risk of gastric cardia/noncardia adenocarcinomas (Shakeri et al., 2015). In addition, prevalence of multiple risk factors for upper gastrointestinal bleeding also is elevated in the elderly population (Kim et al., 2016).

In geriatric patients, *H. pylori* infection can lead to more severe complications, more frequent hospitalizations and higher mortality rates compared with those in younger adults, furthermore, the older subjects often have comorbidity and multidrug therapy (Cizginer et al., 2014).

H. pylori eradication can sharply reduce the long-term risks of the chronic infection such as ulcer bleeding and gastric cancer (Malfertheiner et al., 2007; Malfertheiner et al., 2017). However, the prevalence of *H. pylori* antibiotic resistance, which has been increasing in many countries, is a key reason for eradication failure (Boyanova et al., 2008; De Francesco et al., 2010; Megraud et al., 2013; Malfertheiner et al., 2017).

H. pylori primary resistance is the resistance of strains isolated from patients with no prior treatment attempts for eradication of the infection (Boyanova and Mitov, 2010; Vianna et al., 2016). The aim of the present study was to evaluate *H. pylori* antibiotic resistance in untreated patients aged >65 years *versus* younger adults during the

* Corresponding author at: Tel.: +359-2-91-72-730.
E-mail address: l.boyanova@hotmail.com (L. Boyanova).

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last six years as well as to compare the primary resistance rates in our previous study on *H. pylori* resistance in patients aged ≥60 years in 1996–2003 with those of the patients in the same age group evaluated in 2011–2016.

2. Methods

2.1. Patients and strains

In 2011–2016, antibiotic susceptibility of 233 *H. pylori* strains, involving 62 strains from untreated elderly patients aged 66–93 years (mean age 73.5 years) and 171 strains from untreated younger adults aged 19–64 years (mean age 44.7 years) was evaluated (Table 1). Informed consent was obtained from the patients in the gastroenterology units.

Strain isolation and identification were performed as previously described (Boyanova et al., 2008). The study was approved by the Ethical Committee of Medical University of Sofia, Sofia, Bulgaria.

To compare resistance evolution in the older patients over time, resistance rates in 92 patients aged ≥60 years in 1996–2003 were compared to those in 85 patients in the same age group in 2011–2016 (Boyanova et al., 2003). In the previous study, the *H. pylori* positive patients aged ≥60 years included 28 ulcer patients, 70 non-ulcer patients and 5 gastric cancer patients (Boyanova et al., 2003). The patients evaluated during the last 6 years were aged 60 to 93 years (mean age, 70.3 years) and involved 38 men and 47 women, 17 peptic ulcer and 68 non-ulcer patients.

2.2. Susceptibility testing

Strain susceptibility to clarithromycin and levofloxacin was tested by E test (MIC Evaluator, Oxoid, Basingstoke, UK and Liofilchem, Roseto degli Abruzzi, TE, Italy) as previously described (Boyanova et al., 2008). In brief, *H. pylori* suspensions (equivalent to 2–3 McFarland turbidity standards) were inoculated onto blood Mueller-Hinton agar plates (Oxoid, UK). After the plates were dried, E test strips were placed onto the plates and the plates were incubated in microaerophilic conditions (CampyGen, Oxoid, UK) at 37°C for 48–72 h. The minimal inhibitory concentrations (MICs) were read according to the supplier's recommendations.

The strain susceptibility to amoxicillin, metronidazole, and tetracycline was tested by a breakpoint susceptibility testing method with several consecutive antibiotic concentrations (Boyanova et al., 2008). The breakpoint susceptibility testing method was used as an suitable technique since in our previous study, the category agreement between the results of the method and E test or agar dilution method results has been good (>93%) (Boyanova et al., 2008). The antimicrobial agents were obtained from Sigma-Aldrich, St. Louis, Mo. *H. pylori* suspensions equivalent to 2–3 Mc Farland turbidity standards were inoculated onto Mueller-Hinton agar plates (Oxoid, UK) with 5% sheep blood, containing 0.12, 0.25 and 0.5 mg/L amoxicillin or 4, 8, 16, and 32 mg/L metronidazole, or 1 and 2 mg/L tetracycline. The plates were incubated in microaerophilic conditions (CampyGen, Oxoid, UK) at 37 °C for 2–3 days. Control strains used for the susceptibility testing methods were as previously described (Boyanova et al., 2008).

Resistance rates of *H. pylori* strains from 62 untreated elderly patients aged >65 years in 2011–2016 were compared to those of 171 other adults by both EUCAST breakpoints for *H. pylori* and previously used breakpoints (EUCAST). Non-selective medium plates were used as a control of strain viability. Clarithromycin and levofloxacin MICs were calculated.

As in our prior publication, primary *Helicobacter pylori* resistance rates were evaluated in strains from 92 patients aged ≥60 years in 1996–2003, the rates were compared to those in 85 patients in the same age group in 2011–2016 (Boyanova et al., 2003). To make possible the comparison between the resistance rates of the strains from these patients' groups, the breakpoints used in the prior study were employed. For the same reason, the resistance evolution was evaluated by the previously used breakpoints.

The EUCAST resistance breakpoints for *H. pylori* were: >8 mg/L metronidazole, >0.5 mg/L clarithromycin, >0.125 mg/L amoxicillin, >1 mg/L tetracycline and >1 mg/L levofloxacin (EUCAST). The previously used breakpoints were: >8 mg/L metronidazole, >1 mg/L clarithromycin, >0.5 mg/L amoxicillin, >4 mg/L tetracycline and >1 mg/L ciprofloxacin/levofloxacin (Boyanova et al., 2003).

2.3. Statistical analysis

Chi-square test and Fisher's exact test of independence were performed to compare variables of interest. Differences between groups were considered statistically significant if the p value was <0.05.

3. Results

In 2011–2016, amoxicillin resistance rate in the elderly patients aged >65 years was evaluated by both EUCAST breakpoints and previous breakpoints and was 1.6 and 0%, respectively. Resistance rates by both breakpoint systems were the same to metronidazole (35.5%), clarithromycin (22.6%), tetracycline (1.6%), levofloxacin (32.3%) and metronidazole + clarithromycin (8.1%). There were no significant differences between the resistance rates of the strains from the patients aged >65 years and those from the younger adults ($p \geq 0.196$), (Table 2).

Table 1
Elderly patients and other adults evaluated in 2011–2016.

Patient group	Patients aged >65 years	Other adults	Patients aged ≥60 years	Other adults
Mean age (Range)	73.5 (66–93)	44.7 (19–64)	70.3 (60–93)	42.0 (19–59)
Sex				
Men	31	67	38	60
Women	31	104	47	88
Disease				
Duodenal ulcer	10	24	13	21
Gastric ulcer	3	5	4	4
Duodenal ulcer + gastric ulcer	0	1	0	1
Chronic gastritis	33	111	47	97
GERD[a]	8	21	11	18
Chronic gastritis + GERD	8	9	10	7
Total	62	171	85	148

[a] GERD-gastroesophageal reflux disease.

Table 2
H. pylori resistance rates in untreated elderly patients aged >65 years and other adults in 2011–2016.

Agent	Patient group	Strains tested (n)	Resistance by PBP[a] (%)	*P* value	Resistance by EBP[b] (%)	*P* value
Amoxicillin	Elderly	62	0 (0)	0.576	1 (1.6)	0.685
	Other adults	171	4 (2.3)		7 (4.1)	
Metronidazole	Elderly	62	22 (35.5)	0.501	22 (35.5)	0.501
	Other adults	171	69 (40.4)		69 (40.4)	
Clarithromycin	Elderly	62	14 (22.6)	0.687	14 (22.6)	0.505
	Other adults	171	43 (25.1)		46 (26.9)	
Tetracycline	Elderly	62	1[c] (1.6)	1.000	1 (1.6)	1.000
	Other adults	171	2[c] (1.2)		4 (2.3)	
Levofloxacin	Elderly	62	20 (32.3)	0.196	20 (32.3)	0.196
	Other adults	152	36 (23.7)		36 (23.7)	

[a] PBP, Previous breakpoints used: >8 mg/L metronidazole, >1 mg/L clarithromycin, >0.5 mg/L amoxicillin, >4 mg/L tetracycline and >1 mg/L ciprofloxacin/levofloxacin.
[b] EBP, Breakpoints of resistance according to EUCAST: >8 mg/L metronidazole, >0.5 mg/L clarithromycin, >0.12 mg/L amoxicillin, >1 mg/L tetracycline and >1 mg/L levofloxacin.
[c] tetracycline MIC >2 mg/L.