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RESEARCH ARTICLE

Investigation of multidrug-resistant *Helicobacter pylori* in pediatric patients: A Bulgarian study and literature data

LYUDMILA BOYANOVA[1*], PETYO HADZHIYSKI[2], RUMYANA MARKOVSKA[1] and RAINA GERGOVA[1]

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

[2] Specialized Hospital for Active Pediatric Treatment, Medical University of Sofia, Sofia, Bulgaria

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ABSTRACT

Antibiotic resistance of *Helicobacter pylori* strains from 106 symptomatic children was evaluated according to EUCAST breakpoints and rate of multidrug resistance (MDR) was analyzed. Overall resistance rates were amoxicillin 7.5%, metronidazole 25.5%, clarithromycin 34.0% and ciprofloxacin 14.1%. There were no significant differences in resistance rates according to patients' age (2–6 and 7–18 years) and sex. Combined resistance rate was 19.8%, including double, triple, and quadruple resistance in 13.2% (14 strains), 5.7% (6) and 0.9% (1) of the strains, respectively. MDR was found in 5.9% (5/84) of the children with gastritis and in two of the four children with celiac disease. The MDR was present in three children aged 4–6 years and in four children aged 10–17 years. The total MDR rate (6.6%) in Bulgarian children in 2012–2021 was higher than those in other studies based on EUCAST breakpoints such as those in pediatric patients in Slovenia in 2011–2014 (3.8%), Lithuania in 2013–2015 (0%) and Spain in 2014–2019 (0%), although being lower than those (20.7% in the untreated and 47.0% in the treated children) in China in 2019. In brief, it is of concern that MDR can strongly limit the choice of *H. pylori* therapy of one out of fifteen Bulgarian children and that overall resistance to both metronidazole and clarithromycin can hinder the treatment of 15.1% of the pediatric patients. Susceptibility-guided tailored eradication therapy of *H. pylori* infection should be more frequently implemented in the symptomatic children to avoid risks of both the infection itself and multiple antibiotic treatments.

KEYWORDS

Helicobacter pylori, children, antibiotic, resistance, multidrug

INTRODUCTION

Helicobacter pylori is the cause of one of the most frequent chronic infections in humans [1, 2]. The infection is most often acquired during childhood and may carry a constant risk of developing peptic diseases and tumors over a lifetime. Due to the high prevalence (>50% in Southern and Eastern Europe as well as Asia and South America) and the carcinogenic potential of the bacteria, eradicating the infection is of high importance [2, 3]. Antibiotic resistance is the major reason for eradication failure however, since 2010 a considerable decline in efficacy of eradication success has been observed worldwide [4]. In a multicenter study in untreated adult patients from 18 European countries, only 43% of the strains were fully susceptible to the 6 antibiotics appropriate for eradication of the infection [4]. Double and multidrug resistance in *H. pylori* strongly limits the choice of treatment regimens [5]. Primary MDR has most often been <10% in Europe and the overall or post-therapeutic MDR has been >20–30% in some studies [5].

The aim of the study was to assess double and multidrug (MDR) resistance of *H. pylori* strains from children over 10 years and to compare the data with those in other

*Corresponding author. Department of Medical Microbiology, Medical University of Sofia, Zdrave Street 2, 1431, Sofia, Bulgaria.
E-mail: l.boyanova@hotmail.com

studies from different countries. For this purpose, we included references corresponding to the key words "*H. pylori*", "children", "antibiotic", "resistance" and/or "multidrug resistance" in English, using PubMed, Scopus and Google Scholar data.

MATERIAL AND METHODS

We encompassed data of *H. pylori* strains from 106 children, of them 47 boys and 59 girls, isolated during the routine laboratory diagnostic work in 2012–2021. Eleven children were aged 2–6 years and 90 children were aged 7–18 years. For 5 children, no data about the age were available. Informed written consent was taken from all children' parents.

The pediatric patients suffered from acute gastritis (49) chronic gastritis (35), gastroesophageal reflux disease (GERD, 7), Crohn disease (4), Crohn disease and celiac disease (1), ulcerative colitis (1), liver diseases (4), celiac disease (3), esophageal dyskinesia (1) and gastrointestinal polyposis (1).

Table 1. Double and multidrug resistance in 106 *H. pylori* strains from pediatric patients since 2012

Resistance	Antibiotics	No. of resistant	% of resistant	95% CI
Double	MTZ + CLR	10	9.4	4.9–17.1
	CLR + LVX	1	0.9	0.05–5.9
	MTZ + LVX	2	1.9	0.33–7.3
	AMO + MTZ	1	0.9	0.05–5.9
Multidrug	AMO + MTZ + CLR	3	2.8	0.7–8.6
	MTZ + CLR + LVX	2	1.9	0.33–7.3
	AMO + CLR + LVX	1	0.9	0.05–5.9
	AMO + MTZ + CLR + LVX	1	0.9	0.05–5.9
Total rates	Double	14	13.2	7.7–21.5
	Triple	6	5.7	2.3–12.4
	Quadruple	1	0.9	0.05–5.9
	MDR (triple and quadruple)	7	6.6	2.9–13.6

AMO – amoxicillin, MTZ – metronidazole, CLR – clarithromycin, TET – tetracycline, LVX-levofloxacin.

Strain isolation and identification were carried out by direct staining of the biopsy specimens, rapid urease test and culture as described in our previous study [6].

Antibiotic susceptibility was tested as described in a previous publication by the breakpoint susceptibility test (BST) using blood Mueller-Hinton agar with 5% of sheep blood and the following agents: metronidazole 4, 8 and 16 mg l^{-1}, clarithromycin 0.25, 0.5, 1, 2 and 4 mg l^{-1}, amoxicillin 0.12, 0.25, 0.5, 1 and 2 mg l^{-1}, ciprofloxacin 1 and 10 mg l^{-1}, and tetracycline 1 and 2 mg/l. The BST was performed and the control strains were used as previously described [7]. The susceptibility of the multidrug resistant strains was also confirmed by *E* tests (Roseto degli Abruzzi, TE, Italy).

Reading was made following microaerophilic incubation of the plates with CampyGen envelopes (Oxoid, Basingstoke, UK) at 37 °C for 2–3 days. The resistance breakpoints of EUCAST used were: >8 mg l^{-1} metronidazole, >0.5 mg l^{-1} clarithromycin, >0.12 mg l^{-1} amoxicillin, >1 mg l^{-1} ciprofloxacin/levofloxacin and >1 mg l^{-1} tetracycline [8].

Statistical analysis

Differences between patient groups were determined with chi square with the exact Fisher test for independence. In addition, 95% confidence intervals were also calculated.

RESULTS

Among all 106 strains, resistance rates were amoxicillin 7.5% (8 strains), metronidazole 25.5% (27), clarithromycin 34.0% (36), tetracycline 0.0% (0) and ciprofloxacin as a marker for levofloxacin 14.1% (15). No significant differences in resistance rates were found between the girls and the boys ($P > 0.178$) or between children aged 2–6 years and 7–18 years ($P > 0.158$).

Double and multidrug resistance rates are presented in Table 1. The double resistance rates (to two antibiotics of different classes) were 13.2% (14/106 strains) with the most common double resistance to both metronidazole and clarithromycin (71.4%, 10/14).

Multidrug resistance rate was 6.6% (7/106 strains), involving triple resistance of 5.7% (6/106) and quadruple resistance of 0.9% (1/106) strains (Table 2). The youngest

Table 2. Multidrug resistant *Helicobacter pylori* strains from Bulgarian children over 10 years

Year	Sex	Age (years)	Disease	Antibiotics (MIC mg l^{-1})				
				Amo	MTZ	CLR	TET	LVX
2012	Male	17	Chronic gastritis	R (2)	R (256)	R (64)	S (≤1)	S (0.06)
2013	Female	12	Chronic gastritis	S (0.03)	R (32)	R (128)	S (0.06)	R (6)
2016	Male	4	Celiac disease	S (0.032)	R (256)	R (1.5)	S (≤1)	R (2)
2017	Male	6	Celiac disease	R (0.5)	R (128)	R (3)	S (0.25)	S (0.25)
2018	Female	10	Chronic gastritis	R (0.5)	S (0.023)	R (12)	S (≤1)	R (32)
2019	Male	15	Chronic gastritis	R (0.19)	R (256)	R (256)	S (0.094)	R (≥32)
2021	Female	4	Acute gastritis	R (1)	R (32)	R (8)	S (1)	S (0.125)

MIC- minimal inhibitory concentration, S-susceptible, R-resistant.
AMO – amoxicillin, MTZ – metronidazole, CLR – clarithromycin, TET – tetracycline, LVX-levofloxacin.

Table 3. Combined resistance in *Helicobacter pylori* from children in different countries

Country	Years of study	Patients' groups (years)	No. of children	Method	Breakpoints according to	Double resistance, No. of strains	Double resistance, % of strains	No of MDR strains	% of MDR strains	Reference
Bulgaria	2012–2021	2–18	106	BST, *E* tests	EUCAST	14	13.2	7	6.6 (5.7 triple, 0.9 quadruple)	The present study
China	2019	5–17, untreated	53	*E* test	EUCAST, >0.25 for AMO	27	50.9	11	20.7	18
China	2019	5–17, treated	34	*E* test	EUCAST, >0.25 for AMO	10	29.4	16	47.0	18
China	2012–14	2–16	29 with CLR resistant strains	*E* tests and PCR	Specified	NA	NA	22	75.9	16
Iran	2015–18	5–16	48	*E* test	Specified	25	52.1	20 (triple), 2 (quadruple)	41.7 (triple), 4.2 (quadruple)	15
Israel	2013–15	1–18, untreated and treated	95 untreated, 28 treated	*E* tests	Specified	9 (all strains), 4 (untreated), 5 (treated)	7.3 (all strains), 4.2 (untreated), 17.9 (treated)	0	0.0	12
Italy	2011–12	3–16	66	*E* tests	Specified	5 (in 1998-99), 3 (in 2011-12	8.0 (in 1998-99), 7.0 (in 2011-12	0	0.0	10
Lithuania	2013–15	<18	55	*E* tests	EUCAST	1	8.3	0	0.0	14
Slovenia	2011–14	2–18, untreated	104–107	GDM	EUCAST	18 (3/107, 15/104)	2.8 (of 107 strains), 14.4 (of 104 strains tested)	4 (of 104 strains)	3.8	11
Spain	2014–19	5–17, untreated and treated	80	*E* test	EUCAST	13	16.3	0	0.0	17

BST-breakpoint susceptibility test, GDM-Gradient-diffusion method, DDM-disk diffusion method, ADM-agar dilution method.
MIC- minimal inhibitory concentration, S-susceptible, R-resistant.
AMO – amoxicillin, MTZ – metronidazole, CLR – clarithromycin, TET – tetracycline, LVX-levofloxacin, NA-non available.

children with MDR strains were aged 4 (two children) and 6 years (one child). The older children with MDR *H. pylori* strains were aged 10–17 years. The strains with MDR were detected, one yearly, in 2012, 2013, 2016, 2017, 2018, 2019 and 2021.

In four of the seven MDR strains, triple resistance to amoxicillin, metronidazole and clarithromycin was found.

The MDR was found in four children with chronic gastritis, two of the four children with celiac disease and in a child with acute gastritis. The strain with quadruple resistance to amoxicillin (minimal inhibitory concentration, MIC, 0.19 mg l^{-1}), metronidazole (MIC, ≥256 mg l^{-1}), clarithromycin (MIC, ≥256 mg l^{-1}) and levofloxacin (MIC, ≥32 mg l^{-1}) was isolated from a child aged 15 years suffering from chronic gastritis in 2019.

DISCUSSION

MDR depends on national antibiotic consumption in the given country, individual antibiotic consumption of the patient, patient's comorbidity and characteristics of the infecting strain [5]. In Bulgaria, the national consumption (DDD per 1,000 inhabitants per day) of macrolides, lincosamides and streptogramins (J01F, 3.82 DDD), quinolones (J01M, 2.86 DDD) and cephalosporins (J01D, 4.11 DDD) was among the highest in the European Union in 2017 [9].

In our previous publication, the category agreement between the BST and *E* test or agar dilution method results was high (93.3–100%), therefore we used the BST as a version of the agar dilution method, which is suitable for routine diagnostics of microaerophilic bacteria such as *H. pylori* [7]. *E* tests were used to determine the MICs of the multidrug resistant strains, with resistance to at least 3 antibiotics of different classes.

MDR rates in *H. pylori* in pediatrics have widely varied from 0 to >41% [10–18] (Table 3). However, the comparison of the results is difficult since different breakpoints for resistance were used in different studies (Table 3).

The MDR rate (6.6%) in Bulgarian children determined according to the EUCAST breakpoints was higher than those in other studies also based on EUCAST breakpoints such as those in Slovenia in 2011–2014 (3.8%), Lithuania in 2013–2015 (0%) and Spain in 2014–2019 (0%), [11, 14, 17]. The MDR prevalence in our study was much lower than those (20.7% in the untreated and 47.0% in the treated children) in China in 2019, which were also detected by EUCAST breakpoints with the exception of the breakpoint for amoxicillin resistance (>0.25 mg l^{-1}), [18]. In another study in China, Zhang et al. [16] found frequent (75.9%) MDR in 29 clarithromycin resistant strains evaluated by *E* tests and PCR in 2012–2014.

Two of the four children with celiac disease had MDR *H. pylori* strains. One of these children had frequent respiratory tract infections and otitis. The other child had surgery for an inguinal hernia. The link between previous antibiotic use and celiac disease has been observed [19] and might influence *H. pylori* resistance in children as well.

Eradication success of *H. pylori* in pediatrics has widely varied from 60 to 97.8% and in the review article of Mišak et al. [20], the success was low (60–70%) in 5 of 11 studies. Although we have no data about the eradication rate of *H. pylori* infection in Bulgarian children, the results of the present study imply the need of a wider use of tailored therapy according to the strain susceptibility testing and of improvement of the present and search of newer eradication regimens.

For children with MDR strains, a high-dose of proton pump inhibitor (PPI) plus amoxicillin with a possible addition of a bismuth compound can be used [21, 22]. In Israel, a regimen with amoxicillin, metronidazole, and bismuth subcitrate for 7 days provided eradication in 80% of 45 children evaluated in the study of Shamaly et al. [21]. Importantly, despite the in vitro metronidazole resistance in *H. pylori*, a prolonged regimen with amoxicillin 75 $mgkg^{-1}$day, metronidazole 25 $mgkg^{-1}$day and esomeprazole 1.5 $mgkg^{-1}$day for 2 weeks eradicated 2/3 of *H. pylori* strains resistant to both metronidazole and clarithromycin of the 62 German children evaluated [23].

Vonoprazan has been reported to provide higher eradication success compared with proton pump inhibitors in eradication regimens of *H. pylori* infection [24]. Vonoprazan is an acid-stable and quickly absorbed oral potassium-competitive acid blocker (P-CAB), which was used in a triple regimen containing 20 mg P-CAB, 750 mg amoxicillin, and 200 mg clarithromycin twice a day for 7 days in Japanese junior high school students, providing eradication in 81.3% (by intention-to-treat analysis] and 85.7 (with per protocol analysis), [25]. However, further studies are needed to assess the safety of the P-CAB in children and adolescents [26]. To treat MDR *H. pylori* strains in both children and adults, new agents and regimens should be evaluated.

CONCLUSION

Acquisition of *H. pylori* most often occurs in childhood and the infection is chronic and frequently life-long if not eradicated [20]. According to the present results, it is of concern that the overall combined resistance may affect approximately one out of five children and that MDR may hamper the treatment of more than 6% of the pediatric patients. Moreover, MDR was detected in both younger and older children as well as in both girls and boys. The choice of eradication regimen should be tailored according to the antibiotic susceptibility pattern of the infecting strain or, if this is not possible, it should at least correspond to the resistance rates of *H. pylori* in the country or region. New approaches should be explored to address therapy of MDR *H. pylori* infections.

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