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 10–18 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 treatment regimens [5]. Primary MDR has most often been <10% in Europe and the overall or post-therapeutic MDR has been 15.1% in 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
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’s parents.

The pediatric patients suffered from acute gastritis (49) chronic gastritis (35), gastroesophageal reflex 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

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


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

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 Misak 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 mg kg⁻¹day, metronidazole 25 mg kg⁻¹day and esomeprazole 1.5 mg kg⁻¹day 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 lifelong 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 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.

Conflicts of interest: The authors declare that there are no conflicts of interest.

**Funding information:** The study was supported by the Grant with Contract № D-118 /04.06.2021, Project № 7914 /
19.11.2020 of Medical University of Sofia: Determination of multidrug resistance of *H. pylori* strains in Bulgarian children.

**REFERENCES**


