® Postepy Hig Med Dosw (online), 2014; 68: 1015-1021
e-ISSN 1732-2693

www.phmd.pl
Original Article

Received: 2013.11.18
Accepted: 2014.06.10
Published: 2014.08.22

Prevalence of *Helicobacter pylori cagA, vacA, iceA, babA2* genotypes in Polish children and adolescents with gastroduodenal disease*

Częstość występowania genów wirulecji *cagA, vacA, iceA, babA2* wśród szczepów *H. pylori* izolowanych od dzieci i młodzieży z chorobami przewodu pokarmowego

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Monika Maria Biernat[1, A, B, C, D, E, F], **Grażyna Gościniak**[1, A, D, G], **Barbara Iwańczak**[2, B]

[1] Department of Microbiology, Wrocław Medical University, Poland
[2] 2nd Department of Pediatrics, Gastroenterology and Nutrition, Wrocław Medical University, Poland

Summary

Introduction: Infection with *Helicobacter pylori* is a major cause of chronic gastritis and peptic ulcer disease in children and its consequences in adulthood can lead to serious complications, including in particular the development of gastric cancer. Our aim was to analyze the relationship between the occurrence of selected genes such as *cagA*, *vacA*, *iceA*, and *babA2* determining pathogenicity of *H. pylori* strains and clinical outcome in children.

Material and methods: The study was performed on *H. pylori* strains isolated from biopsies taken from 130 children and adolescents with non-ulcer dyspepsia (NUD), gastric and duodenal ulcers (PUD) and gastroesophageal reflux disease (GERD). Genes such as *cagA*, *vacA* (allelic variants: *s1/s2*, *m1/m2*), *iceA* (allelic variants: *iceA1*, *iceA2*) and *babA2* were determined by polymerase chain reaction (PCR).

Results: The *cagA* gene was detected in 79/130 (60.8%) *H. pylori* isolates. The presence of the *cagA* gene was significantly associated with duodenal ulcer ($p<0.05$). The *vacAs1/m1* genotype was more frequent in children with ulcers than in other groups, whereas the *vacAs2/m2* genotype was more frequent in patients with gastritis and GERD. The *iceA1*, *iceA2* and *babA2* genes were present in 59/130 (45.4%), 27/130 (21%) and 30/130 (23.1%) of the strains, respectively. The *vacAs1/cagA+* genotype was most frequently observed in strains isolated from children with PUD. The predominant genotype in children with NUD and GERD was *vacAs2/cagA-/iceA1+/babA2-*.

Conclusion: The study showed a high incidence of strains with increased virulence, possessing *cagA*, *vacAs1* and *iceA1* genes in symptomatic children with *H. pylori* infection.

Keywords: ***Helicobacter pylori*** • **children** • **adolescens** • **genotypes**

*This work was made possible by the grant support of the Ministry of Science and Higher Education, No. 3230/P01/2007/32, Poland.

Full-text PDF: http://www.phmd.pl/fulltxt.php?ICID=1118211

Word count: 2512
Tables: –
Figures: –
References: 30

Author's address: Monika Maria Biernat M.D., Ph.D., Department of Microbiology, Chałubinskiego Street 4, 50-368 Wrocław; e-mail: mobiernat@gmail.com

Abbreviations: **GERD** – gastroesophageal reflux disease, **NUD** – non-ulcer dyspepsia, **PCR** – polymerase chain reaction, **PUD** – gastric and duodenal ulcer disease

Introduction

Helicobacter pylori infection usually occurs in childhood and is a major cause of chronic gastritis and peptic ulcer disease in children. The consequences of infection occur in adulthood, and lead to many diseases in the gastrointestinal tract, including in particular the development of gastric cancer. *H. pylori* infection is present in 93% of children with duodenal ulcer and in 56% of gastric ulcers, although these disorders in the pediatric population are rare [2]. Moreover, the clinical course and the nature of inflammation are different from those seen in adults [23,29]. In developed countries, the incidence of *H. pylori* infection in children is less than 12% and shows a tendency to decrease, while in developing countries it may exceed 40% [11]. In Poland, the incidence of infection in the pediatric population is about 32% and varies depending on the region [14]. The risk of developing serious diseases of the upper gastrointestinal tract in the course of *H. pylori* infection is affected by factors dependent on the host, environmental conditions and strain-specific virulence factors [30]. Increased virulence of *H. pylori* bacilli is associated with the presence of certain virulence factors, mainly protein CagA and VacA cytotoxin. Research carried out in the Polish population shows that the presence of these factors among *H. pylori* strains is very high, especially in children [7]. Additionally, the clinical course of infection caused by this microorganism may be affected by other factors encoded by the following genes: *iceA*, *babA*, *oipA*, *dupA*, *homB* [17,20,24]. According to some authors, phenotypic and genotypic diversity of *H. pylori* strains may influence the degree and intensity of the individual inflammatory response and results in a variety of clinical manifestations in both children and adults [1,3]. In Poland, studies on the role of genotypes of *H. pylori* strains in children so far are rare. The study of the relationship between genotype and clinical manifestations in children may contribute to a better understanding of the pathogenesis of *H. pylori* infection and thus provide a basis for the development of methods to prevent these diseases in adulthood. The aim of this study was to characterize the genotype of *H. pylori* strains isolated from children with diseases of the upper gastrointestinal tract, and to analyze the association between the prevalence of selected genes such as *cagA*, *vacA* (*allele s1/s2*, *s1a/s1b*, *m1/m2*), *iceA* (*iceA1/iceA2*), *babA2* and the clinical course of *H. pylori* infection.

Material and methods

This retrospective study was performed in the years 2008-2012 on *H. pylori* strains collected in the Department of Microbiology, Wrocław Medical University. The strains were isolated from biopsies taken from 130 children and adolescents (66 girls and 64 boys) between 4 and 18 years old who were referred to the Second Department of Pediatric Gastroenterology and Nutrition, Wrocław Medical University in the years 2007-2012 for evaluation of upper gastrointestinal signs and symptoms (recurrent abdominal pain, dyspepsia, vomiting). The patient population was homogeneous, of European origin, residing in the Lower Silesian district of Poland. Patients who had undergone gastroduodenoscopy and had been diagnosed with *H. pylori* infection were included in the study. The exclusion criteria included previous diagnosis of celiac disease, inflammatory bowel disease, or allergy, evidence of severe pathologies or intestinal parasites. Subjects who had taken antibiotics or an acid suppressant during the previous one month were also excluded. This study was approved by the ethics committee of Wroclaw Medical University, Poland (Approval No. KB-350/2006) and was undertaken according to the Declaration of Helsinki. Informed written consent was obtained from all parents of children and from patients older than 16 years of age participating in the study. At least two biopsy specimens from the antrum and in the case of pathologic changes also from the corpus were taken from each child during upper gastroduodenal endoscopy. One was used for histopathological examination and was evaluated histologically according to the Updated Sydney System [6]. The other biopsies were sent for microbiological analysis. Three groups of patients were evaluated: the first comprised children (n= 78) with non-ulcer dyspepsia (NUD), in whom endoscopy revealed esophagitis, gastritis, or duodenitis. The second group consisted of patients (n= 30) with peptic ulcer diseases (PUD) (peptic and/or duodenal ulcer disease). The

third group comprised children with gastroesophageal reflux disease (GERD) (n=22). The diagnosis of *H. pylori* infection was based on bacteriological and histological examination. The gastric biopsies were cultured as previously described [13]. Briefly, the strains were identified as *H. pylori* by Gram stain morphology, positive culture and positive catalase, oxidase and urease tests. Subsequently, the bacteria were cultured on Columbia agar medium (Difco) with horse blood (7%) and on Columbia agar medium (Difco) with horse blood and a selective supplement (Dent), containing vancomycin 10 mg/l, trimethoprim 10 mg/l, cefsulodin 5 mg/l and amphotericin B 5 mg/l for 3 days at 37°C under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2). After the primary isolation and identification, the strains were kept frozen at -70°C in Brucella broth containing 15% glycerol. Subsequently, the bacteria were cultivated in the same conditions as primary cultures. On one plate a colony of bacteria was collected and subsequently passaged on two plates with the same medium. The plates were again incubated in microaerophilic conditions. Chromosomal DNA was extracted from *H. pylori* culture samples using the Genomic Mini kit (A&A Biotechnology, Poland) according to the manufacturer's instructions. Briefly, colonies of *H. pylori* from two plates of 10^8 CFU were scraped and transferred into centrifuge tubes containing 100 µl of sterile phosphate-buffered saline. Suspensions were vortexed vigorously, then transferred into tubes containing 100 µl of Tris buffer (10 mM Tris-HCl pH 8.5), 200 µl of lysis buffer and 20 µl of proteinase K. The tubes were incubated at 37°C for 20 min and transferred to a water bath at 75°C for 5 min, then centrifuged at 14 000 x g. DNA was eluted and stored at -20°C. Analysis of the *vacA*, *babA2* and *iceA1* genes was performed by multiplex PCR whereas the *cagA* and *iceA2* genes were detected by single PCR. In all cases the PCR reactions were performed using a Taq PCR core kit (Qiagen). On the basis of published sequences primers were synthesized and used for different amplification reactions [8]. The 16s rRNA *H. pylori* reference strain J99 was used as an internal control. For detection of the *cagA* gene amplification was performed in 20 µl reaction mixtures containing 0.25 µM of *cagA* complementary primers, and Taq PCR core kit (Qiagen). Products were amplified under the following conditions: 5 min at 94°C for initial denaturation followed by 35 cycles of 45 s at 94°C, 45 s at 62°C, and 45 s at 72°C with a final round of 5 min at 72°C. For detection of *vacA* s and m regions the amplification reaction was performed in 20 µl reaction mixtures containing approximately: 0.25 µM of *vacA* s/m and 16s rRNA complementary primers, 3.5 mM of $MgCl_2$, 100 ng of genomic DNA, and 5 U of Taq polymerase in a standard buffer. The reaction included an initial denaturation of target DNA at 94°C for 5 min, and then 35 cycles at 94°C for 1 min, 57°C for 1 min, 72°C for 1 min, then finally 72°C for 10 min. For analysis of *iceA1* and *babA* genotypes amplification was carried out in 20 µl volumes containing 0.25 µM of each primer and the Taq PCR core kit (Qiagen), whereas for *iceA2* analysis the 20 µl reaction mixture consisted of 0.5 µM of complementary primers, 3.5 mM of $MgCl_2$, 100 ng of genomic DNA, and 2 U of Taq polymerase in a standard buffer. Cycling conditions were 35 cycles at 94°C for 1 min, 45°C for 1 min, 72°C for 1 min, then finally 72°C for 10 min. All PCR reactions were performed in a PTC-200 thermocycler (MJ RESEARCH). PCR products were analyzed using 2.5% agarose gel electrophoresis. SYBR Green I (Lonza Bioscience) was used as a DNA binding fluorescence dye. A strain isolated from a patient with gastritis, possessing both *iceA1* and *babA2* genes, was used as a positive control. For detection of *iceA2* reference strain J99 was used as a positive control.

Statistical analyses

Differences among groups were tested using the chi-square, McNemar, Fisher, and Yates tests. $P< 0.05$ was considered as statistically significant. Statistical analyses were done by using STATISTICA Version 10.0.

Results

The prevalence of virulence genes among children and adolescents analyzed in the study is present in Table 1. Of the 130 examined patients, infection with more than one *H. pylori* strain, suggesting mixed infection, was detected in 16 (12.3%) cases. In these patients isolated strains possessed different alleles of the gene *vacA* (*vacA m1 + m2* or *vacAs1/m1 + s2*) in 11 cases (9 patients with NUD, 1 with PUD and 1 with GERD) or both alleles of the gene *iceA* (*iceA1 + iceA2*) in 5 cases (respectively 3 with NUD and 2 with GERD). The *cagA* gene was present among more than 60% of the strains, and was statistically significantly more frequent among strains isolated from patients with PUD (p<005). The most common alleles of the gene *vacA* were *s1* (62.3%) and *m2* (67%), but the dominant alleles were *s2/m2* (37%) (p <0.05). The *iceA1*, *iceA2* and *babA2* genes were present in 59/130 (45.4%), 27/130 (21%) and 30/130 (23.1%) of the strains, respectively. The distribution of complex genotypes among strains isolated from children with different clinical diagnoses is shown in Table 2. Patients with mixed infection were eliminated from this analysis. Among the strains isolated from children with peptic ulcer disease, the most frequently observed genotype was *vacAs1/cagA*. Among patients with NUD and GERD the predominant genotype of *H. pylori* strains was *vacAs2/cagA-/iceA1+/babA*.

Discussion

The study analyzed the genotypes of *H. pylori* in children with digestive tract diseases. There are few reports concerning molecular epidemiology of *H. pylori* strains isolated from Polish children. High frequency of the *cagA* gene in the Polish population of children and adults was reported by other authors [7]. High prevalence of the *cagA* gene in children has also been noted by researchers from other centers worldwide [9,12,26]. In contrast, some other authors reported dominance of *cagA*-negative strains in children in southern Euro-

Table 1. Prevalence of *H. pylori* virulence genes detected in examined strains

Type of gene	NUD (n=78)	PUD (n=30)	GERD (n=22)	Total (n=130)
cagA+	45(57.7%) p=0.383	20(66.7%) p < 0.05*	14(63.6%) p<0.0001*	79(60.8%)
cagA-	33(43.2%)	10(33.3%)	8(36.3%)	51(39.2%)
vacA s1	47(60.2%)	20(66.7%)	14(63.6%)	81(62.3%)
vacA s2	31(39.7%)	10(33.3%)	8(36.3%)	49(37.7%)
vacA m1	24(30.8%)	11(36.7%)	7(31.8%)	42(32.3%)
vacA m2	54(69.2%) p<0.0001*	19(63.3%) p < 0.05*	*15(68.1%) p=0.07*	88(67.7%)
vacA s1/m1	24(30.8%)	10(33.3%)	7(31.8%)	41(31.5)
vacA s1/m2	22(28.2%)	10(33.3%)	7(31.8%)	39(30%)
vacA s2/m2	32(41%)	10(33.3%)	8(36.3%)	50(38.5%)
babA+	14(18%) p < 0.05*	9(30%) p >0.05	7(31.8%) p >0.05	30(23.1%)
babA-	64(82%)	21(70%)	15(68.1%)	100(77%)
iceA1+	*38(48.7%) p<0.001**	11(36.7%) p < 0.05*	10(45.4%) p>0.05	59(45.4%)
iceA2+	14(18%) p<0.001*	9(30%) p < 0.05*	4(18.1%) p>0.05	27(20.7%)
iceA1+ /iceA2+	3(3.8%)	0	2(9.1%)	5(3.9%)
iceA1-/iceA2-	23(29.5%)	10(33.3%)	6(27.2%)	39(30%)

*Values are considered as statistically significant.
NUD- non-ulcer dyspepsia, PUD-peptic ulcer disease, GERD- gastroesophageal reflux disease

pe [21]. The high percentage of *cagA*-positive strains of the Polish population in our study may indicate an increased risk of development of stomach and duodenal ulcers as well as precancerous lesions in the course of *H. pylori* infection. On the other hand, these strains appear to be easier to eradicate [27]. It was found that the differences in the occurrence of *vacA* alleles in the examined groups were statistically significant. These results correlate with studies in children and adults in other Polish regions [16]. The presence of *H. pylori* infection in our area caused by *vacAs1* strains that have the ability to produce VacA cytotoxin proves the high virulence of the strains tested. In children with peptic ulcer disease, most strains had *vacA s1/m2* and *s1/m1 vacA* allele variants while in patients with GERD and NUD there occurred strains with less capacity to produce toxins, or those that did not produce it at all, which is consistent with the observations of other authors [12,18]. The *iceA1* allele was more frequent in both strains isolated from children with PUD and with NUD (p<0.05). In addition, the *iceA1* allele was more frequent among strains isolated from children with duodenal ulcer, while the *iceA2* allele was dominant among strains isolated from children with peptic ulceration. The role of the gene *iceA* is not fully known and the results obtained from different centers are contradictory [4,12]. It is supposed

Table 2. Combined *cagA*, *vacA*, *babA* and *iceA* genotypes in correlation with clinical outcome

Genotype	NUD (n=66)	PUD (n=29)	GERD (n=19)	Total (n=114)
vacAs1/cagA+/iceA1+/babA+	4(6.1%)	2(6.9%)	1(5.3%)	7(6.1%)
vacAs1/cagA+/iceA1+/babA-	13(19.7%)	4 (13.8%)	3(15.8%)	20(17.5%)
vacAs1/cagA+/iceA2+/babA+	2(3.1%)	1(3.4%)	1(5.3%)	4(3.5%)
vacAs1/cagA+/iceA2+/babA-	6(9.1)	4(13.8%)	1(5.3%)	11(9.6%)
vacAs1/cagA+/iceA-/babA+	3(4.5%)	3(10.3%)	2(10.5%)	8(7%)
vacAs1/cagA+/iceA-/babA-	6(9.1%)	4(13.8%)	4(21.1%)	14(12.2%)
vacAs1/cagA-/iceA1+/babA+	0	1(3.4%)	0	1(0.9%)
vacAs1/cagA-/iceA1+/babA-	1(1.5%)	0	0	1(0.9%)
vacAs1/cagA-/iceA2+/babA-	0	1(3.4%)	0	1(0.9%)
vacAs1/cagA-/iceA-/babA+	1(1.5%)	0	0	1(0.9%)
vacAs1/cagA-/iceA-/babA-	1(1.5%)	0	0	1(0.9%)
vacAs2/cagA+/iceA-/babA+	0	1(3.4%)	0	1(0.9%)
vacAs2/cagA+/iceA1/babA-	2(3.1%)	1(3.4%)	0	3(2.6%)
vacAs2/cagA+/iceA2/babA-	1(1.5%)	0	0	1(0.9%)
vacAs2/cagA-/iceA1+/babA-	16(24.2%)	2(6.9%)	5(26.3%)	23(20.1%)
vacAs2/cagA-/iceA2+/babA+	0	0	1(5.3%)	1(0.9%)
vacAs2/cagA-/iceA2+/babA-	4(6.1%)	3(10.3%)	1(5.3%)	8(7%)
vacAs2/cagA-/iceA-/babA+	1(1.5%)	0	0	1(0.9%)
vacAs2/cagA-/iceA-/babA-	5(7.5%)	2(6.9%)	0	4(3.5%)
Total	66(100%)	29(100%)	19(100%)	114(100%)

that the *iceA1* allele may be responsible for adherence to epithelial cells, and causes an increase in concentration of IL-8 in gastric mucosa. Our results are similar to data from a meta-analysis of the role of *iceA* gene alleles on clinical outcome [25]. It seems that the *iceA1* allele is more common in symptomatic children in our area and may be associated with more severe clinical course of infection. The occurrence of mixed infection with *iceA*-type strains can lead to high genetic variability of *H. pylori* during long-term colonization, as well as the ability to exchange genetic material between different strains [15]. The presence of multiple strains isolated from one host has been reported [7,22]. Another gene analyzed in the study, *babA2*, is less common in our examined strains than in strains isolated from symptomatic patients in Bulgaria and Turkey [4,8]. Moreover, a similar frequency of the *babA2* gene was detected in the genetic material in strains from children with PUD and GERD, but it was detected much less frequently among strains from children with NUD ($p<0.05$). The results of our analysis indicate a higher incidence of the *babA2* gene among strains from children with duodenal ulcer than with gastric ulcer. Some authors demonstrated on a gerbil model differences between strains with high and low *babA2* gene expression in the induction of severe mucosal injury and inflammatory host response [19]. It can be assumed that the *babA2* genotype is important in distinguishing *H. pylori*-related gastroduodenal diseases and the severity of inflammation dependent on *babA2* may predict clinical outcome [5,28]. However, the different associations between the b*abA2* genotype and gastric and duodenal ulcers should be further investigated in the future.

In this study, we analyzed the distribution of complex genotypes among strains isolated from children with different clinical diagnoses. Such analyses have not been conducted previously in children in Poland. Among the strains isolated from children with peptic ulcer disease the most frequently observed genotype was *vacAs1/cagA*, but in some of them (13%) the genes *iceA* and *babA* were also detected. It is possible that these genes may enhance virulence of strains and have an impact on the nature of the inflammatory changes in the stomach. A positive correlation between the presence of genotype *babA2/cagA+/vacAs1* and incidence of duodenal ulcers, inflammation of the stomach cardia, gastric cancer and MALT lymphoma was reported [10]. Among patients with NUD and GERD the predominant genotype of *H. pylori* strains was *vacAs2/cagA-/iceA1+/babA*, which is considered to be less virulent. The role of the *iceA1* gene requires further study. It seems that analysis of a strain's genotype is more adequate in correlation with clinical outcome than determination of separate genes. The analysis in this study is retrospective in nature; furthermore, it was conducted on strains isolated from children from one clinical center. Strains from different regions of the country may possess different genotypes.

Conclusions

In summary, the study showed a high incidence of strains with increased virulence, possessing *cagA*, *vacAs1* and *iceA1* genes in a cohort of pediatric patients with *H. pylori* infection. Large differences in the prevalence of complex genotypes of *H. pylori* strains in children may indicate a variety of strains of *H. pylori*, and also create the need for continuing research on the correlation of specific virulence factors and their role in the course of infection in children.

Acknowledgements

We thank Dr Łukasz Łaczmański from the Laboratory of Molecular Research, Department of Endocrinology and Diabetology, Wroclaw Medical University for assistance in the development of PCR methods for detection of *H. pylori* genotypes.

References

[1] Amieva M.R., El-Omar E.M.: Host-bacterial interactions in *Helicobacter pylori* infection. Gastroenterology, 2008; 134: 306-323

[2] Bąk-Romaniszyn L., Płaneta-Małecka I.: Therapeutic failures in *Helicobacter pylori* eradication. Ped. Wsp. Gastroenterol. Hepatol. Żyw. Dz., 2004; 6: 387-391

[3] Blaser M.J.: Heterogeneity of *Helicobacter pylori*. Eur. J. Gastroenterol. Hepatol., 2012; 9 (Suppl. 1): S3-S6

[4] Boyanova L., Yordanov D., Gergova G., Markovska R., Mitov I.: Association of iceA and babA genotypes in *Helicobacter pylori* strains with patient and strain characteristics. Antonie Van Leeuwenhoek, 2010; 98: 343-350

[5] Chen M.Y., He C.Y., Meng X., Yuan Y.: Association of *Helicobacter pylori* babA2 with peptic ulcer disease and gastric cancer. World J. Gastroenterol., 2013; 19: 4242-4251

[6] Dixon M.F., Genta R.M., Yardley J.H., Correa P.: Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am. J. Surg. Pathol., 1996; 20: 1161-1181

[7] Dzierżanowska-Fangrat K., Crabtree J.E., Rożynek E., Dura W., Celińska-Cedro D., Wojda U., Dzierżanowska D.: *Helicobacter pylori* cagA genotype and density of colonization in relation to gastric inflammation in children. Eur. J. Gastroenterol. Hepatol., 2002; 14: 1303-1307

[8] Erzin Y., Koksal V., Altun S., Dobrucali A., Aslan M., Erdamar S., Dirican A., Kocazeybek B.: Prevalence of *Helicobacter pylori* vacA, cagA, cagE, iceA, babA2 genotypes and correlation with clinical outcome in Turkish patients with dyspepsia. Helicobacter, 2006; 11: 574-580

[9] Garcia G.T., Aranda K.R., Gonçalves M.E., Cardoso S.R., Iriya K., Silva N.P., Scaletsky I.C.: High prevalence of clarithromycin resistance and cagA, vacA, iceA2, and babA2 genotypes of *Helicobacter pylori* in Brazilian children. J. Clin. Microbiol., 2010; 48: 4266-4268

[10] Gerhard M., Lehn N., Neumayer N., Boren T., Rad R., Schepp W., Miehlke S., Classen M., Prinz C.: Clinical relevance of the *Helicobacter pylori* gene for blood-group antigen binding adhesin. Proc. Natl. Acad. Sci. USA, 1999; 96: 12778-12783

[11] Homan M., Hojsak I., Kolaček S.: *Helicobacter pylori* in pediatrics. Helicobacter, 2012; 17 (Suppl. 1): 43-48

[12] Homan M., Luzar B., Kocjan B.J., Orel R., Mocilnik T., Shrestha M., Kveder M., Poljak M.: Prevalence and clinical relevance of cagA, vacA, and iceA genotypes of *Helicobacter pylori* isolated from Slovenian children. J. Pediatr. Gastroenterol. Nutr., 2009; 49: 289-296

[13] Iwańczak B., Biernat M., Iwańczak F., Grabińska J., Matusiewicz K., Gościniak G.: The clinical aspects of *Helicobacter heilmannii* infection in children with dyspeptic symptoms. J. Physiol. Pharmacol., 2012; 63: 133-136

[14] Iwańczak F., Iwańczak B.: Treatment of *Helicobacter pylori* infection in the aspect of increasing antibiotic resistance. Adv. Clin. Exp. Med., 2012; 21: 671-680

[15] Maciorkowska E., Roszko I., Kaczmarski M., Kowalczuk O., Chyczewski L.: Occurrence rate of *Helicobacter pylori* iceA alleles in infected patients in the Białystok Province. Przegl. Epidemiol., 2008; 62 (Suppl. 1): 69-74

[16] Maciorkowska E., Roszko I., Kowalczuk O., Kaczmarski M., Chyczewski L., Kemona, A.: The evaluation of vacA gene alleles frequency in *Helicobacter pylori* strains in children and adults in Podlaskie region. Folia Histochem. Cytobiol., 2007; 45: 215-219

[17] Matteo M.J., Armitano R.I., Granados G., Wonaga A.D., Sánches C., Olmos M., Catalano M.: *Helicobacter pylori* oipA, vacA and dupA genetic diversity in individual hosts. J. Med. Microbiol., 2010; 59: 89-95

[18] Miciuleviciene J., Calkauskas H., Jonaitis L., Kiudelis G., Tamosiunas V., Praskevicius A., Kupcinskas L., Berg D.: *Helicobacter pylori* genotypes in Lithuanian patients with chronic gastritis and duodenal ulcer. Medicina, 2008; 44: 449-454

[19] Ohno T., Vallström A., Rugge M., Ota H., Graham D.Y., Arnqvist A., Yamaoka Y.: Effects of blood group antigen-binding adhesin expression during *Helicobacter pylori* infection of Mongolian gerbils. J. Infect. Dis., 2011; 203: 726-735

[20] Oleastro M., Cordeiro R., Ferrand J., Nunes B., Lehours P., Carvalho-Oliveira I., Mendes A.I., Penque D., Monteiro L., Mégraud F., Ménard A.: Evaluation of the clinical significance of *homB*, a novel candidate marker of *Helicobacter pylori* strains associated with peptic ulcer disease. J. Infect. Dis., 2008; 198: 1379-1387

[21] Oleastro M., Gerhard M., Lopes A.I., Ramalho P., Cabral J., Sousa Guerreiro A., Monteiro L.: *Helicobacter pylori* virulence genotypes in Portuguese children and adults with gastroduodenal pathology. Eur. J. Clin. Microbiol. Infect. Dis., 2003; 22: 85-91

[22] Patra R., Chattopadhyay S., De R., Ghosh P., Ganguly M., Chowdhury A., Ramamurthy T., Nair G.B., Mukhopadhyay A.K.: Multiple infection and microdiversity among *Helicobacter pylori* isolates in a single host in India. PLoS One, 2012; 7: e43370

[23] Raymond J., Bergeret M., Kalach N.: *Helicobacter pylori* infection in children. Presse Med., 2008; 37: 513-518

[24] Romo-Gonzalez C., Salama N.R., Burgeno-Ferreira J., Ponce-Castaneda V., Lazcano-Ponce E., Camorlinga-Ponce M., Torres J.: Differences in genome content among *Helicobacter pylori* isolates from patients with gastritis, duodenal ulcer, or gastric cancer reveal novel disease-associated genes. Infect. Immun., 2009; 77: 2201-2211

[25] Shiota S., Watada M., Matsunari O., Iwatani S., Suzuki R., Yamaoka Y.: *Helicobacter pylori* iceA, clinical outcomes, and correlation with cagA: a meta-analysis. PLoS One, 2012; 7: e30354

[26] Sicinschi L.A., Correa P., Bravo L.E., Peek R.M.Jr., Wilson K.T., Loh J.T., Yepez M.C., Gold B.D., Thompson D.T., Cover T.L., Schneider B.G.: Non-invasive genotyping of *Helicobacter pylori* cagA, vacA, and hopQ from asymptomatic children. Helicobacter, 2012; 17: 96-106

[27] Sugimoto M., Yamaoka Y.: Virulence factor genotypes of *Helicobacter pylori* affect cure rates of eradication therapy. Arch. Immunol. Ther. Exp., 2009; 57: 45-46

[28] Talarico S., Gold B.D., Fero J., Thompson D.T., Guarner J., Czinn S., Salama N.R.: Pediatric *Helicobacter pylori* isolates display distinct gene coding capacities and virulence gene marker profiles. J. Clin. Microbiol., 2009; 47: 1680-1688

[29] Uhlig H.H., Tannapfel A., Mössner J., Jedwilayties S., Deutscher J., Müller D.M., Kiess W., Richter T.: Histopathological parameters of *Helicobacter pylori*-associated gastritis in children and adolescents: comparison with findings in adults. Scand. J. Gastroenterol., 2003; 38: 701-706

[30] Yamaoka Y.: Mechanisms of disease: *Helicobacter pylori* virulence factors. Nat. Rev. Gastroenterol. Hepatol., 2010; 7: 629-641

The authors have no potential conflicts of interest to declare.