ACTA MICROBIOLOGICA BULGARICA Volume 34 / 1 (2018)

Review

Main Characteristics of *Helicobacter pylori* Strains and Infection in Bulgaria

Lyudmila Boyanova

Department of Medical Microbiology, Medical University of Sofia, Sofia, Bulgaria

Abstract

The aim of the work was to summarize some specific characteristics of Helicobacter pylori strains and infection in Bulgaria according to our studies. Data of our studies, mostly those from the last decade are discussed. Infection seroprevalence dropped from 82.6% to 72.4% in adult blood donors over 18 years, being only 24.2% in children. Virulence genes were frequent (>80%) in strains from symptomatic patients for vacuolating cytotoxin (vacA) s1a and cytotoxin-associated gene A (cagA) and outer inflammatory protein (oipA) in active status. In Bulgaria, vacA i alleles were more useful to determine the strain virulence compared with other vacA alleles. Using EUCAST breakpoints, overall H. pylori resistance rates were: metronidazole 33.8%, clarithromycin 28.1%, levofloxacin 19.4%, amoxicillin 4.0% and tetracycline 3.7%. Multidrug resistance was found in 4.2% of untreated and more often (15.4%) in treated adults, and in 1.2% of untreated children. Risk factors, resistance evolution and extended anamnesis are discussed. Lactobacillus delbrueckii subsp. bulgaricus strains inhibited the growth of a portion of H. pylori strains, including those resistant to antibiotics. Neutralized filtrates of 7 strains suppressed the growth of >2/3 of H. pylori strains. There was an association between regular honey consumption and a lower infection rate, and an inverse association between honey and yoghurt intake and anti-CagA antibodies against virulent strains. In conclusion, although the infection prevalence decreases in younger people, still the high infection prevalence, common virulence genes, increasing resistance to clarithromycin and quinolones and multidrug resistance show that H. pylori infection problem in our country is not yet fully resolved.

Keywords: *Helicobacter pylori*, seroprevalence, virulence, antibiotic resistance, multidrug, non-antibiotic, dietary

Резюме

Цел на работата беше да се обобщят някои специфични характеристики на *Helicobacter pylori* щамовете и инфекцията в България според нашите изследвания. Обсъдени са данните от нашите проучвания, предимно тези от последното десетилетие. Серопревалирането на инфекцията спадна от 82.6% на 72.4% при донори на възраст 18 г., като беше само 24.2% при децата. Гените за вирулентност бяха чести (>80%) при щамовете от симптоматични пациенти за вакуолизиращия цитотоксин (*vacA*) sla и цитотоксин-асоциирания ген А (*cagA*), както и на външния протеин на възпалението (*oipA*) в активен статус. В нашата страна, *vacA* і алелите бяха по-полезни за определяне на щамовата вирулентност в сравнение с другите *vacA* алели. С граничните стойности на EUCAST, общите честоти на резистентност на *H. pylori* бяха: metronidazole 33.8%, clarithromycin 28.1%, levofloxacin 19.4%, amoxicillin 4.0% и tetracycline 3.7%. Множествена резистентност беше намерена при 4.2% от нелекуваните и по-често (15.4%) при лекуваните възрастни, както и при 1.2% при нелекуваните деца. Дискутирани са рисковите фактори, еволюцията на резистентността и разширената анамнеза. Щамовете *Lactobacillus delbrueckii* subsp. *bulgaricus* инхибираха растежа на част от *H. pylori* щамовете, включително тези, резистентни към антибиотици. Неутрализираните филтрати от 7 щама потиснаха растежа на >2/3 от *H. pylori* щамовете. Имаше връзка между редовната консумация на мед и по-ниската честота на инфекцията и обратната връзка между консумацията на мед и кисело

мляко и анти-CagA антителата срещу вирулентните щамове. В заключение, въпреки че честотата на инфекцията намалява при по-младите хора, все още нейното разпространение, честите гени на вирулентност, повишаващата се резистентност към clarithromycin и хинолони и множествената резистентност показват, че проблемът с *H. pylori* инфекцията у нас все още не е напълно решен.

Introduction

Helicobacter pylori is an important and frequent pathogen for humans and is closely associated with chronic gastritis, duodenal and gastric ulcers, gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma (Kusters *et al.*, 2006; Bagheri *et al.*, 2013; Pellicano *et al.*, 2016). The bacteria have an arsenal of numerous virulence factors and associated genes such as *cag* pathogenicity island (*cag*PAI)- a complex of many genes including cytotoxin-associated gene A (*cagA*) and cytotoxin-associated gene E (*cagE)*, vacuolating cytotoxin *(vacA),* outer inflammatory protein (*oipA*), duodenal ulcer promoting **(***dupA)* gene, blood group antigen binding adhesin (*babA2),* outer-membrane protein *homB* and others (Shiota *et al.*, 2013; Kalali *et al.*, 2014).

The bacteria infect more than half of the world's population and the bacterial species is considered as a class I carcinogen (the group of most powerful carcinogens) by the World Health Organization (Wroblewski *et al.*, 2010).

H. pylori seroprevalence

The prevalence of *H. pylori* infection has been found to widely vary, being higher in the developing countries compared with the developed countries (Mentis *et al.*, 2015). High infection prevalence (>50-70%) was found in South America, Africa and Asia, and lower prevalence has been reported in North America and northern Europe (30-40%), (Kusters *et al.*, 2006, Eusebi *et al.*, 2014; Garza-Gonzalez *et al.*, 2014; Mentis *et al.*, 2015). In Southeastern Europe, *H. pylori* prevalence has frequently been >50% and high (>82%) rates have been found in Portugal and Turkey (Eusebi *et al.*, 2014).

In Bulgaria, *H. pylori* seroprevalence has decreased, although rather slowly (from 82.6 to 72.4%), over 18 years among adult blood donors (Fig. 1). The prevalence of both *H. pylori* antibodies and antibodies against cytotoxin-associated gene A protein (CagAIgG) indicating virulent strains (in about 2/3 of the donors) was high (Yordanov *et al.*, 2016). However, among the asymptomatic children aged 1-17 years, only 24.2% were positive for the infection, and CagA seroprevalence among the positive was 40.0% (Yordanov *et al.*, 2017a). This shows a decrease in the infection due to improved socio-economic conditions and frequent antibiotic use, but on the other hand, the problem of infection in our country remains not fully resolved.

Seroprevalence was linked to age, female sex, number of siblings and mother's educational level (Yordanov *et al.*, 2016).

Oral *H. pylori*

Given that oral-oral transmission is one of the most important transmission routes of the transmission of the infection, the increasing interest in oral *H. pylori* has been justified (Adler *et al.*, 2014).

In our study, the frequency of oral *H. pylori* was very low (2.3%), however, in the oral cavity of a child with both gingivitis and gastritis, a highly virulent (*cagA+*/*vacA* s1 type) *H. pylori* strain with a double resistance (to metronidazole and clarithromycin) was isolated and evaluated by 4 methods: culture, immunofluorescent microscopy using monoclonal antibodies, PCR and antibiotic susceptibility testing (Boyanova *et al.*, 2013a). The oral strains can be a potential source of infection or reinfection with virulent and antibiotic resistant strains.

Although the role of oral *H. pylori* is still a controversial topic, Bharath *et al.* (2014) have reported the presence of the same strain simultaneously in the supragingival plaque and gastric mucosa. In a review article, it has been suggested that the oral cavity may be an important *H. pylori* reservoir and can be associated with the relapse of the infection (Adler *et al.*, 2014).

H. pylori virulence gene arsenal

H. pylori is the causative agent of chronic gastritis, peptic ulcers, gastric cancer and MALT lymphoma (Wroblewski *et al.*, 2010). The bacteria can display a huge number of virulence genes and mechanisms. A high frequency of *H. pylori* virulence genes was detected in Bulgarian symptomatic patients such as:

- >88% for vacuolating cytotoxin (*vacA)* s1a allele,
- >80% for *cagA* (cytotoxin-associated gene A) and *oipA* (outer inflammatory protein) in ac-

tive or "on" status,

- >68% for *cagE* (cytotoxin-associated gene E) and *iceA1* (induced by contact with epithelium) genes and
- >48% for *babA2* (blood group antigen binding adhesin A) genes (Boyanova *et al.*, 2009b; Boyanova *et al.*, 2010b; Boyanova *et al.*, 2011; Markovska *et al.*, 2011).

Virulent *cagA*+ and *vacA* s1 strains were common in our country and the ulcer patients most often had strains with virulent genotypes.

Importantly, in contrast to *vacA* s1 and m1, *vacA* i1 was more frequent (75.0%) in patients with peptic ulcer versus non-ulcer patients (58.6%), and genotyping can be useful in the detection of virulent strains that require a much more aggressive therapy than the other strains (Yordanov *et al.*, 2012).

An association between *vacA* i1 and the active "on" status of *oipA* gene was detected (Markovska *et al.*, 2011). Other genes such as *homB* are not recommended for routine diagnostic purposes (unpublished data).

The severity and outcome of *H. pylori*-related diseases most often are associated with both presence and integrity of the *cag* pathogenicity island (*cag*PAI of about 30 genes), which injects CagA and peptidoglycan into the host epithelial cells, leading to high IL-8 release (Wroblewski *et al.*, 2010).

In our study, *cagE*+ was found to gradually increase with patient's age, indicating either general or individual dynamics in strain virulence (Boyanova *et al.*, 2011). Therefore, the clinical outcome of the infection is due to the cumulative activity of *H. pylori* virulence genes. Although both *cagA* and *cagE* genes were associated with the severity of the diseases, *cagE* was found to be more useful to distinguish the virulent strains from the less virulent strains compared with the *cagA* (Boyanova *et al.*, 2011).

For practical purposes, it is important to use an extra primer pair to increase *cagA* gene detection by >14%, which can be of benefit in countries where *cagA* positive strains are frequent (Boyanova *et al.*, 2011).

H. pylori resistance to antibiotics

H. pylori resistance to all antibiotics used for eradication of the infection has been reported and higher primary resistance rates to clarithromycin (≥20%) and quinolones (≥20%) has been detected in developed countries compared with the developing regions, while higher rates to metronidazole (>75%) have been observed in the developing countries (Megraud *et al.*, 2013; Mascellino *et al.*, 2017). Furthermore, post-treatment resistance (>50-80%) after unsuccessful therapy has been much higher than primary resistance (Van Zanten *et al.*, 2010).

Some reported factors associated with primary resistance rates have been national outpatient

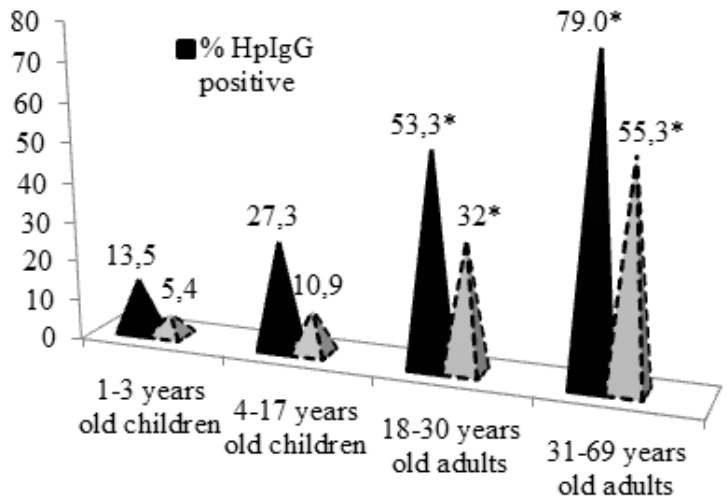

Fig. 1. Seroprevalence (%) of *H. pylori* and anti-CagA IgG antibodies (Yordanov *et al.*, 2016, Yordanov *et al.*, 2017a).

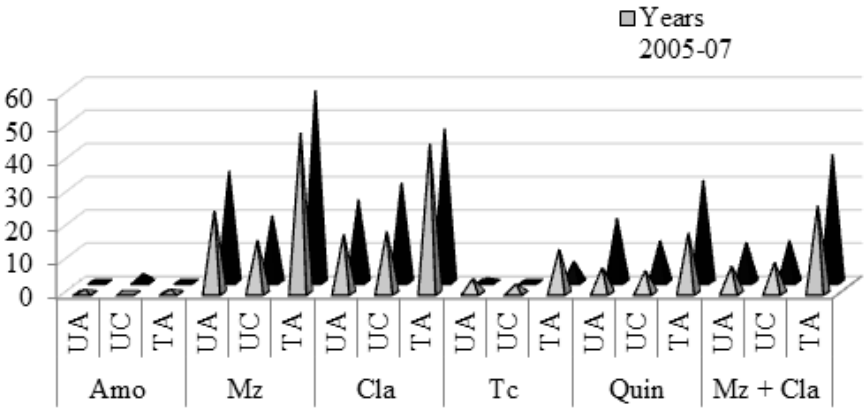

Fig. 2. Changes in *H. pylori* resistance rates from 2005-07 to 2010-15 (Boyanova *et al.*, 2008; Boyanova *et al.*, 2016a)

antibiotic consumption; patients' sex, age, ulcer or non-ulcer disease, strain virulence etc. (Megraud *et al.*, 2013; Boyanova *et al.*, 2016a).

In Bulgaria, using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints, the overall resistance rates were: amoxicillin 4.0%, metronidazole 33.8%, clarithromycin 28.1%, levofloxacin 19.4%, tetracycline 3.7%, and rifampin 8.3% (EUCAST, 2015; Boyanova *et al.*, 2016a), (Fig. 2).

There was a constant increase in the total primary clarithromycin resistance (1.4-fold from 2005-07 to 2010-15), (Boyanova *et al.*, 2016a). Moreover, in 2010-2015, an increased primary resistance to metronidazole (1.3-fold) and fluoroquinolones (2.4-fold) has been detected (Boyanova *et al.*, 2016a).

In elderly patients, the primary quinolone resistance rate (30.0%) in 2011-2016 was much (3.4-fold) higher than that (8.9%) found in the same age group of patients in 1996-2003 (Boyanova *et al.*, 2017b).

Post-treatment problems have been: frequent (≥50%) *H. pylori* resistance in adults to metronidazole or clarithromycin; double (metronidazole + clarithromycin) resistance in 42.3% as well as multidrug resistance (Boyanova *et al.*, 2016a).

An association was found between A2143G mutation for clarithromycin resistance (often predicting eradication failure of the triple clarithromycin-based treatment regimens) and the less virulent *vacA* i2 strains, and vice versa, between the A2142G mutation and the more virulent *vacA* i1 strains, which may explain the reported more successful eradication of the more virulent strains (Boyanova *et al.*, 2016b).

Some risk factors for resistance revealed by logistic regression were: birthplace in towns for metronidazole resistance, healthcare profession for metronidazole and metronidazole + clarithromycin resistance, hospital centers for clarithromycin resistance, non-ulcer disease for metronidazole resistance rate (28.3%) vs. ulcer disease (17%) and living in Sofia for metronidazole resistance vs. living elsewhere, probably suggesting the influence of the population density (Boyanova *et al.*, 2009a; Boyanova *et al.*, 2013b). In the elderly patients, the factors associated with *H. pylori* resistance can be linked to the most frequent comorbidity and antibiotic treatment for non-*pylori* infections as well as with the national consumption of antibiotics (Boyanova *et al.*, 2017b).

Prior (10 years ago) national tetracycline consumption evaluated as a resistance factor has demonstrated that the decrease in the resistance rates can take many years (Boyanova, 2009; Boyanova *et al.*, 2016a).

The benefits of a long-term analysis of the evolution of primary resistance of *H. pylori* were established. The increase in quinolone resistance was predicted by extending the study period to 20 years (Boyanova *et al.*, 2010a). Long-term follow-up can also be recommended for other infections.

Multidrug resistance

Multidrug resistance that can be defined as resistance to three or more antibiotics belonging to different classes, and primary multidrug resistance have been infrequent (0.4-6%) in Europe and higher in countries on other continents, e.g. 10% in China (Biernat *et al.*, 2014; Mourad-Baars *et al.*, 2015; Shi *et al.*, 2016).

Fig. 3. Non-antibiotic agents showing potential in the control of *H. pylori* infection according to our studies: yoghurt, green/black tea and honey.

In our studies, an alarming multidrug resistance was found in *H. pylori* as early as in 2000, and in 2005 to 2008, a worrying multidrug resistance was observed in 4.2% of the untreated, and more often (15.4%), in treated adults and even in 1.2% of the untreated children (Boyanova, 2009).

In our recent study on primary *H. pylori* resistance in elderly patients over 20 years (from 1996-2003 to 2011-2016), either a double or a triple antibiotic resistance was detected in 21.0% of the subjects aged >65years (Boyanova *et al.*, 2017b).

Complex reasons for multidrug resistance were found, involving the increased national consumption of MLS (macrolide/lincosamide/streptogramin) group of antibiotics in Bulgaria and that of quinolones since 2000, the increasing primary *H. pylori* resistance to clarithromycin, high tetracycline consumption in 1994-1999 and, in some single cases, use of non-standard regimens, such as azithromycin-containing regimes, or nitroimidazole reuse (Boyanova, 2009; Boyanova *et al.*, 2016a). These results call attention to the need for a very strict application of the antibiotic policy, regular monitoring of resistance rates and correct choice of treatment eradication regimes.

It is alarming that a fivefold resistance (perhaps linked to efflux pumps) to 5 antibiotics was found in a strain from a treated man with chronic gastritis and GERD (Boyanova *et al.*, 2014).

Pan-European Registry on *H. pylori* management: (Hp-EuReg)

I have had the honor to be selected as a Local coordinator for Bulgaria in the Pan-European Registry on *H. pylori* management: (Hp-EuReg). Some important results of the huge study encompassing 20000 patients were (McNicholl *et al.*, 2014; McNicholl *et al.*, 2015; McNicholl *et al.*, 2016a; McNicholl *et al.*, 2016b):

- overall *H. pylori* eradication success with the first and second line regimens was found to be unsatisfactory (74%),
- adding esomeprazole as a proton pump inhibitor (PPI) as well as the use of bismuth or non-bismuth quadruple (concomitant) therapies resulted in improved eradication rate,
- acceptable eradication rates (90% by intention to treat- ITT analysis) can be achieved by non-bismuth concomitant therapeutic regimens if they are optimized with a double PPI dose,
- after 3-4 eradication attempts, *H. pylori* resistance to clarithromycin, metronidazole, clarithromycin plus metronidazole, and quinolone resistance was high, varying from 48 to 67%,
- in the so-called rescue regimens (after several failed attempts), it may be useful to extend regimens up to 14 days and to use esomepra-

zole as a PPI,

- side effects of the therapy were found in a proportion (17%) of the patients, but very few (only 4%) of them were discontinued.

The results of the Hp-EuReg are of great benefit to the treatment of the *H. pylori* infection, since the eradication success has decreased (to 50% in some countries) over the years (Fakheri *et al.,* 2014).

Non-antibiotic agents

Some dietary factors have shown anti-*H. pylori* effects and can influence the frequency of the associated infection and perhaps the disease outcome. Among the potentially beneficial non-antibiotic agents have been green tea, probiotics, honey and propolis, cranberry juice, garlic, liquorice and broccoli sprouts, while high-salt and meat consumption have been deemed unfavorable factors (Lin and Koskella, 2015; Mousavi *et al.*, 2016; Bakal *et al.*, 2017).

Probiotics such as *Lactobacillus* spp. can provide different benefits for the control of *H. pylori* infection, involving direct growth inhibition, release of lactic acid and other organic acids and bacteriocins or bacteriocin like inhibitory substances, decrease of bacterial urease activity and bacterial density in the gastric mucosa as well as immunomodulation, and thus can be used to obtain an increase (although slight) in eradication rates and a significant reduction of side effects of the eradication therapy (Lesbros-Pantoflickova *et al.*, 2007; Boltin, 2016).

In our studies, *Lactobacillus delbrueckii* subsp. *bulgaricus* inhibited the growth of a portion of *H. pylori* strains, including those resistant to antibiotics (Boyanova *et al.*, 2009c; Boyanova *et al.*, 2017a). The most active strains suppressed 53% of *H. pylori* strains even at neutralized pH. Bacteriocin-like substances of 7 strains were able to suppress the growth of >2/3 of *H. pylori* strains (Boyanova *et al*, 2017a). Bacteriocins offer a strong advantage in choosing probiotics for *H. pylori* infection.

Both black and green teas (prepared from *Camellia sinensis* leaves) are frequently consumed beverages and also are favorable non-antibiotic agents with potential in the control of *H. pylori* infection due to the polyphenolic catechins, including epigallocatechin gallate (Boyanova *et al.*, 2015). In our study, logistic regression analysis revealed that the risk of *H. pylori* positivity in green/black tea consumers is lower (OR, 0.45; 95% CI, 0.21-0.95) compared with that in the other patients (Boyanova *et al.*, 2015).

Honey has also been a topic of interest. African honey has demonstrated anti- *H. pylori* activity associated with its osmotic effect, hydrogen peroxide and other active substances (Mousavi *et al.*, 2016).

In our study performed with ^{13}C urea breath test, a beneficial effect (OR 0.38; 95% CI, 0.19-0.78) of regular honey consumption (≥1 day/week) on *H. pylori* infection prevalence in untreated dyspeptic patients was observed and confirmed by logistic regression (Boyanova *et al.*, 2015).

The benefits of Bulgarian honey were additionally evaluated and confirmed in another of our studies: evaluating seroprevalence, there was an association between honey consumption (>5 days/week) and a lower infection rate (OR, 0.68, 95% CI, 0.473-0.967), and an inverse association between honey (OR, 0.65; 95% CI, 0.486-0.859) and yoghurt (OR, 0.56; 95% CI, 0.341-0.921) intake and CagA IgG antibodies indicating virulent strains (Yordanov *et al.*, 2017b).

Therefore, regular and frequent yoghurt, green/black tea and honey consumption may be recommended as a not only useful but also pleasant habit for additional control of this common and hronic human infection (Fig. 3).

Conclusion

Although the infection prevalence decreases in younger people, still the high infection prevalence, common virulence genes among the strains as well as their constantly increasing resistance to clarithromycin and quinolones and multidrug resistance show that *H. pylori* infection is a problem that is not yet fully resolved.

References

Adler, I., A. Muiño, S. Aguas, L. Harada, M. Diaz, A. Lence, M. Labbrozzi, J. M. Muiño, B. Elsner, A. Avagnina, V. Denninghoff (2014). *Helicobacter pylori* and oral pathology: relationship with the gastric infection. *World J. Gastroenterol*. **20**: 9922-9935.

Bagheri, N., A. Taghikhani, G. Rahimian, L. Salimzadeh, F. Azadegan Dehkordi, F. Zandi, M. H. Chaleshtori, M. Rafieian-Kopaei, H. Shirzad (2013). Association between virulence factors of *Helicobacter pylori* and gastric mucosal interleukin-18mRNA expression in dyspeptic patients. *Microb. Pathog.* **65**: 7-13.

Bakal, S. N., S. Bereswill, M. M. Heimesaat (2017). Finding novel antibiotic substances from medicinal plants – antimicrobial properties of *Nigella sativa* directed against multidrug-resistant bacteria. *Eur. J. Microbiol. Immunol.* (Bp). **7**: 92-98.

Bharath, T. S., M. S. Reddy, R. Dhanapal, N. G. Raj Kumar, P. Neeladri Raju, T. Saraswathi (2014). Molecular detection and corelation of *Helicobacter pylori* in dental plaque and gastric biopsies of dyspeptic patients. *JOMFP* **18**: 19-24.

Biernat, M. M., E. Poniewierka, J. Błaszczuk, L. Czapla, R. Kempiński, D. Ksiądzyna, J. Grabińska, A. Bińkowska, F. Megraud, G. Gościniak (2014). Antimicrobial susceptibility of *Helicobacter pylori* isolates from Lower Silesia, Poland. *Arch. Med. Sci.* **10**: 505-509.

Boltin, D (2016). Probiotics in *Helicobacter pylori*-induced peptic ulcer disease. *Best Pract. Res. Clin. Gastroenterol.* **30**: 99-109.

Boyanova, L., G. Gergova, R. Nikolov, L. Davidkov, V. Kamburov , C. Jelev, I. Mitov (2008). Prevalence and evolution of *Helicobacter pylori* resistance to 6 antibacterial agents over 12 years and correlation between susceptibility testing methods. *Diagn. Microbiol. Infect. Dis*. **60**: 409-415.

Boyanova, L (2009). Prevalence of multidrug-resistant *Helicobacter pylori* in Bulgaria. *J. Med. Microbiol*. **58**: 930-935.

Boyanova, L., J. Ilieva, G. Gergova, Z. Spassova, R. Nikolov, L. Davidkov, I. Evstatiev, V. Kamburov, N. Katsarov, I. Mitov (2009a). Evaluation of clinical and socio-demographic risk factors for antibacterial resistance of *Helicobacter pylori* in Bulgaria. *J. Med. Microbiol*. **58**: 94-100.

Boyanova, L., R. Markovska, D. Yordanov, M. Marina, K. Ivanova, S. Panayotov, G. Gergova, I. Mitov (2009b). High prevalence of virulent *Helicobacter pylori* strains in symptomatic Bulgarian patients. *Diagn. Microbiol. Infect. Dis.* **64**: 374-380.

Boyanova, L., M. Stephanova-Kondratenko, I. Mitov (2009c). Anti-*Helicobacter pylori* activity of *Lactobacillus delbrueckii* subsp. *bulgaricus* strains: preliminary report. *Lett. Appl. Microbiol.* **48**: 579-584.

Boyanova, L., R. Nikolov, G. Gergova, I. Evstatiev, E. Lazarova, V. Kamburov, E. Panteleeva, Z. Spassova, I. Mitov (2010a). Two-decade trends in primary *H. pylori* resistance to antibiotics in Bulgaria. *Diagn. Microbiol. Infect. Dis.* **67**: 319-326.

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

Boyanova L, D. Yordanov, G. Gergova, R. Markovska, I. Mitov (2011). Benefits of *Helicobacter pylori cagE* genotyping in addition to *cagA* genotyping. A Bulgarian study. *Antonie Van Leeuwenhoek* **100**: 529-535.

Boyanova, L., V. Panov, D. Yordanov, G. Gergova, I. Mitov (2013a). Characterization of oral *Helicobacter pylori* strain by 4 methods. *Diagn. Microbiol. Infect. Dis.* **77**: 287-288.

Boyanova, L., J. Ilieva, G. Gergova, I. Evstatiev, R. Nikolov, I. Mitov (2013b). Living in Sofia is associated with a risk for antibiotic resistance in *H. pylori*. A Bulgarian study. *Folia Microbiol*. **58**: 587-591.

Boyanova, L., L. Davidkov, G. Gergova, N. Kandilarov, I. Evstatiev, E. Panteleeva, I. Mitov (2014). *Helicobacter pylori* susceptibility to fosfomycin, rifampin and five usual antibiotics for *H. pylori* eradication. *Diagn. Microbiol. Infect. Dis.* **79**: 358–361.

Boyanova, L., J. Ilieva, G. Gergova, B. Vladimirov, R. Nikolov, I. Mitov (2015). Honey and green/black tea consumption may reduce the risk of *Helicobacter pylori* infection. *Diagn. Microbiol. Infect. Dis*. **82**: 85-86.

Boyanova, L., G. Gergova, I. Evstatiev, Z. Spassova, N. Kandilarov, P. Yaneva, R. Markovska, I. Mitov (2016a). *Helicobacter pylori* resistance to six antibiotics by two breakpoint systems and resistance evolution in Bulgaria. *Infect. Dis. (Lond)*. **48**: 56-62.

Boyanova, L., R. Markovska, D. Yordanov, G. Gergova, I. Mitov (2016b). Clarithromycin resistance mutations in *Helicobacter pylori* in association with virulence factors and antibiotic susceptibility of the strains. *Microb. Drug Resist*. **22**: 227-232.

Boyanova, L., G. Gergova, R. Markovska, D. Yordanov, I. Mitov (2017a). Bacteriocin-like inhibitory activities of seven *Lactobacillus delbrueckii* subspecies *bulgaricus* strains against antibiotic susceptible and resistant *Helicobacter pylori* strains. *Lett. Appl. Microbiol.* **65**: 469-474.

Boyanova, L., G. Gergova, R. Markovska, N. Kandilarov, L. Davidkov, Z. Spassova, I. Mitov (2017b). Primary *Helicobacter pylori* resistance in elderly patients over 20 years: A Bulgarian study. *Diagn. Microbiol. Infect. Dis.* **88**: 264-267.

EUCAST – European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 5.0, 2015 . Available from: http://www.eucast.org

Eusebi, L. H., R. M. Zagari, F. Bazzoli (2014). Epidemiology of *Helicobacter pylori* infection. *Helicobacter* **19** Suppl **1**: 1-5.

Fakheri, H., Z. Bari, M. Aarabi, R. Malekzadeh (2014). *Helicobacter pylori* eradication in West Asia: A review. *World J. Gastroenterol*. **20**: 10355-10367.

Garza-González, E., G. I. Perez-Perez, H. J. Maldonado-Garza, F. J. Bosques-Padilla (2014). A review of *Helicobacter pylori* diagnosis, treatment, and methods to detect eradication. *World J. Gastroenterol*. **20**: 1438-1449.

Kalali, B., R. Mejías-Luque, A. Javaheri, M. Gerhard (2014). *H. pylori* virulence factors: influence on immune system and pathology. *Mediators Inflamm*. **2014**: 426309. doi: 10.1155/2014/426309.

Kusters, J. G., A. H. van Vliet, E. J. Kuipers (2006). Pathogenesis of *Helicobacter pylori* infection. *Clin. Microbial. Rev.* **19**: 449-490.

Lesbros-Pantoflickova, D., I. Corthésy-Theulaz, A. L. Blum (2007). *Helicobacter pylori* and probiotics. *J. Nutr.* **137**: 812S-818S.

Lin, D., B. Koskella (2015). Friend and foe: factors influencing the movement of the bacterium *Helicobacter pylori* along the parasitism-mutualism continuum. *Evol. Appl.* **8**: 9-22.

Markovska, R., L. Boyanova, D. Yordanov, G. Gergova, I. Mitov (2011). *Helicobacter pylori oipA* genetic diversity and its associations with both disease and *cagA*, *vacA* s, m and i alleles among Bulgarian patients. *Diagn. Microbiol. Infect. Dis*. **71**: 335-340.

Mascellino, M. T., B. Porowska, M. De Angelis, A. Oliva (2017). Antibiotic susceptibility, heteroresistance, and updated treatment strategies in *Helicobacter pylori* infection. *Drug Des. Devel. Ther.* **11**: 2209-2220.

McNicholl, A. G., A. Gasbarrini, B. Tepes, F. Lerang, D. S. Bordin, O. Shvets, T. Rokkas, L. Kupcinskas, M. Leja, M. Katicic, J. C. Machado, L. Boyanova, K. Przytulski, et al (2014). Pan-European registry on *H. pylori* management

(Hp-EuReg): safety. *Helicobacter* **19**: P11.26.

McNicholl, A. G., A. Gasbarrini, B. Tepes, D. S. Bordin, F. Lerang, M. Leja, T. Rokkas, D. Vaira, O. Shvets L. Kupcinskas, A. Perez-Aisa, T. Axon, G. M. Buzas, I. Simsek, M. Katicic, J. C. Machado, V. Lamy, K. Przytulski, L. Boyanova, P. Bytzer, *et al.* (2015). Pan-European registry on *H. pylori* management (HP-EuReg): bacterial resistance. *Gastroenterology* **148**: S-417.

McNicholl, A. G., A. Gasbarrini, B. Tepes, M. Castro-Fernandez, A. P. Aisa, L. Bujanda, J. Molina-Infante, A. Lanas, D. Vaira, T. Rokkas, P. Almela, E. Medina, J. Ortuño, S. Michopoulos, M. Domínguez-Cajal, A. J. Lucendo, J. Barrio, I. Modolell, L. Pozzati, V. Ntouli, J. Perez-Lasala, S. D. Georgopoulos, L. Rodrigo, M. Vujasinovic, D. S. Bordin, J. C. Machado, L. Boyanova, *et al.* (2016a). Pan-European registry on *H. pylori* management (Hp-EuReg): interim analysis of non-bismuth quadruple concomitant treatment. *Gastroenterology* **150**: S875.

McNicholl, A. G., B. Tepes, A. Gasbarrini, A.P. Aisa, D. Vaira, D. S. Bordin, F. Lerang, M. Castro-Fernandez, L. Bujanda, M. Leja, M. Vujasinovic, T. Rokkas, L. Kupcinskas, L. Veijola, O. Shvets, G. M. Buzás, J. C. Machado, L. Boyanova, *et al.* (2016b). Pan-European registry on *H. pylori* management (HP-EuReg): interim analysis of first- and second-line treatments. *Gastroenterology* **150**: S875.

Megraud, F., S. Coenen, A. Versporten, M. Kist, M. Lopez-Brea, A. M. Hirschl, L. P. Andersen, H. Goossens, Y. Glupczynski; Study Group participants (2013). *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. *Gut* **62**: 34- 42.

Mentis, A., P. Lehours, F. Mégraud (2015). Epidemiology and diagnosis of *Helicobacter pylori* infection. *Helicobacter* **20**: 1-7.

Mourad-Baars, P. E., H. F. Wunderink, M. L. Mearin, R. A. Veenendaal, J. M. Wit, K. E. Veldkamp (2015). Low antibiotic resistance of *Helicobacter pylori* in the Netherlands. *Helicobacter* **20**: 69-70.

Mousavi, S. S. B., S. Naghdifar, M. Rafieian-Kopaei (2016). Treatment of helicobacter pylori infection by herbal drugs; a review on current data. *J. Prev. Epidemiol.* **1**: e06.

Pellicano R., D. G. Ribaldone, S. Fagoonee, M. Astegiano, G. M. Saracco, F. Mégraud (2016). A 2016 panorama of *Helicobacter pylori* infection: key messages for clinicians. *Panminerva Med.* **58**: 304-317.

Shi, J., Y. Jiang, Y. Zhao (2016). Promising in vitro and in vivo inhibition of multidrug-resistant *Helicobacter pylori* by linezolid and novel oxazolidinone analogues. *J. Glob. Antimicrob. Resist*. **7**: 106-109.

Shiota, S., R. Suzuki, Y. Yamaoka (2013). The significance of virulence factors in *Helicobacter pylori*. *J. Dig. Dis.* **14**: 341-349.

Van Zanten, S. V., S. Desai, L. Best, G. Cooper-Lesins, D. Malatjalian, D. Haldane, K. Peltekian (2010). Rescue therapy using a rifabutin-based regimen is effective for cure of *Helicobacter pylori* infection. *Can. J. Gastroenterol.* **24**: 303–306.

Wroblewski, L. E., R. M. Jr. Peek, K. T. Wilson (2010). *Helicobacter pylori* and gastric cancer: factors that modulate disease risk. *Clin. Microbiol. Rev*. **23**: 713-739.

Yordanov, D., L. Boyanova, R. Markovska, G. Gergova, I. Mitov (2012). Significance of *Helicobacter pylori vacA* intermediate region genotyping-a Bulgarian study. *Diagn. Microbiol. Infect. Dis*. **74**: 253-257.

Yordanov, D., L. Boyanova, R. Markovska, J. Ilieva, N. Andreev, G. Gergova, I. Mitov (2016). *Helicobacter pylori* seroprevalence in Bulgaria over 18 years and anti-CagA seroprevalence. *C. R. Acad. Bulg. Sci.* **69**: *1651-1658.*

Yordanov, D., L. Boyanova, R. Markovska, P. Hadzhiyski, G. Gergova, I. Mitov (2017a). Seroprevalence of *Helicobacter pylori* IgG and CagA IgG in Bulgarian children. *C. R. Acad. Bulg. Sci.* 2017a, in press.

Yordanov, D., L. Boyanova, R. D. Markovska, J. Ilieva, N. Andreev, G. Gergova, I. G. Mitov (2017b). Influence of dietary factors on *Helicobacter pylori* and CagA seroprevalence in Bulgaria. *Gastroenterol. Res. Pract.* Article ID 9212143, doi:10.1155/2017/9212143