Helicobacter pylori causes substantial morbidity and mortality, and the course of infection results from complex interactions between host, environmental and bacterial factors. It is generally accepted that H. pylori eradication is the best method of treatment for peptic ulcer disease and prevention of its complications. However, the antimicrobial agents used in eradication regimens cause various alterations in gastrointestinal microflora, which can lead to side effects affecting the patient’s compliance. Moreover, antimicrobial therapy is responsible for increasing resistance not only in H. pylori but also in colonising microflora, and, therefore, alternative approaches to the treatment and prevention of H. pylori infection have been investigated.

Key words: Helicobacter pylori, microflora, probiotics, virulence

INTRODUCTION

Helicobacter pylori colonises the gastric mucosa of approximately half of the world’s population. However, the prevalence of infection varies geographically, ranging from 20 to 50% in industrialized countries to over 80% in developing countries (1). Despite such a high frequency of infections, only a minority (10-20%) of affected individuals have clinical manifestations of infection, such as peptic ulcer disease, gastric carcinoma or MALT-lymphoma (2). It is believed that many environmental, bacterial and host-related factors can influence the course of the infection. The most effective treatment for peptic ulcer disease is the eradication of H. pylori (3). However, the efficacy of eradication therapy depends strongly on antimicrobial susceptibility and the patient’s compliance (4).
The prevalence of *H. pylori* resistance changes with time, varying between countries and patient populations, and, therefore, requires constant monitoring. Tolerance to eradication therapy is an important factor affecting the patient’s compliance, and can be improved by the concomitant use of probiotics.

The following sections of this article will discuss *H. pylori* bacteriology with a focus on its virulence factors, microbiological diagnostic approach, current antimicrobial resistance in Poland, influence of eradication regimens on intestinal microflora and the role of probiotics in the prevention and treatment of *H. pylori* infections.

**H. pylori: the bacterium and its virulence factors**

*H. pylori* is a Gram-negative spiral-shaped rod which has developed several mechanisms necessary for its survival and replication in the acidic environment of the stomach. *H. pylori* synthesises urease, an enzyme catalyzing the hydrolysis of urea to ammonia, which alkalises low stomach pH and produces a neutral environment around the bacteria (5). It has been shown that urease activity depends on the pH around *H. pylori*, and it is increased in a lower pH which opens urea channels in the bacterial cell membrane (6). Urease has a toxic effect on gastric epithelial cells (7), and it is a potent immunogen which stimulates a strong immune response. Apart from urease and its product, ammonia, several other bacterial products are involved in the gastric tissue damage. Glycosulfatase, a proteolytic enzyme, degrades mucin and phospholipases A2 and C digest phospholipids in the cell membranes (8, 9). *H. pylori* synthesises superoxide dismutase and catalase which protect it from phagocytosis, and from killing by phagocytic cells (10, 11). The spiral shape and the flagella attached to one pole enables the bacterium to penetrate the mucous layer, and to reach gastric epithelial cells (7). *H. pylori* motility is enhanced at increased media viscosity, and decreased at a pH < 4 (7). *H. pylori* produces several adhesins, e.g. BabA and BabB proteins (blood-group antigen binding adhesions) which bind the bacterium to gastric epithelial cells (12). Adhesion of *H. pylori* to epithelial cells leads to the release of proinflammatory cytokines (such as IL-8) and increased apoptosis of the epithelium (13, 14). Several structures have been proposed as putative gastric receptors for *H. pylori*. Recently, it has been suggested that the CD74 molecule on the gastric epithelial cells can serve as a receptor for *H. pylori*. CD74 is closely associated with class II MHC molecules, which could indicate that the gastric epithelial cells are not only a target of the infection, but can also function as antigen-presenting cells (15).

Among various virulence factors identified in *H. pylori*, the cytotoxin–associated gene A (CagA), vacuolating toxin A (VacA), and BabA2 adhesin have been associated in several studies with enhanced pathogenicity of *H. pylori* and a more severe clinical outcome of the infection (16-18). The cagA gene is a marker of the cag pathogenicity island (cag PAI), which includes several genes involved in bacterial colonisation and gastric inflammatory response. CagA
is present in about 60-70% of *H. pylori* strains isolated in western countries (19). It has been shown that the CagA positive strains induce a higher production of proinflammatory cytokines in the gastric mucosa, and in some populations an infection with such strains is linked with an increased risk of peptic ulcer disease, gastric atrophy and gastric cancer (19-21). Additionally, the strains possessing the *cagA* gene have been associated with denser colonisation of the gastric mucosa in both adults and children, and the bacterial load has been found to correlate with gastric neutrophil and lymphocyte infiltration (22, 23).

About half of *H. pylori* strains produce vacuolating cytotoxin (VacA), responsible for epithelial cell vacuolation and death, although nearly all of them harbour the *vacA* gene. The *vacA* gene has a mosaic structure and cytotoxin production is related to the composition of allelic types of the signal sequence (s1a, s1b, s1c, s2) and middle region (m1, m2) of the gene.

The strains possessing s1 allele synthesise a functional VacA toxin, whereas s2 positive strains have little or no cytotoxic activity (17). Moreover, strains of the s1m1 genotype are more virulent than s1m2 strains and linked with more severe forms of the disease (17). Additionally, although the expression of VacA does not require the functional *cagA* gene, *vacA* s1m1 genotype and thus VacA cytotoxin activity strongly correlates with the presence of the *cagA* gene (24). Over half of all *H. pylori* strains isolated in Poland are CagA positive and harbour the more toxigenic s1 allele of *vacA* gene (25).

*H. pylori* exposed to unfavourable conditions, such as nutrient starvation or growth inhibitors (e.g. some antibiotics, bismuth or proton pump inhibitors) transforms into unculturable coccoid forms which have been reported to survive for a long time in the environment. It has been suggested that these dormant forms can be involved in infection transmission, failure of eradication therapy, and the recurrence of the disease (26).

**Bacteriological diagnosis of *H. pylori* infections**

Various invasive and non-invasive tests have been used for the diagnosis of *H. pylori* infection. Culture of the bacterium and subsequent susceptibility testing require endoscopy and biopsy of the gastric mucosa. In untreated patients, *H. pylori* is most frequently isolated from the stomach antrum, whereas in individuals taking antisecretory drugs (such as H2 antagonists or proton pump inhibitors) high numbers of bacteria can be also found in the corpus of the stomach. In patients with duodenitis or duodenal ulcer, *H. pylori* is more often isolated from the antral part of the gastric mucosa than from the *duodenum*. Therefore, to obtain reliable culture results it is recommended to take at least one biopsy from the antral mucosa and two biopsies from the *corpus*, whereas duodenal or esophageal biopsies (in patients with duodenitis or esophagitis) are of a lesser importance (27). It should be noted that the *H. pylori* colonisation of gastric mucosa can be patchy, which can lead to discrepant results of diagnostic tests such as culture, histology and urease
test (28). *H. pylori* has also been isolated from the focci of gastric metaplasia in Meckel’s diverticulum, esophagus, urinary bladder, or rectum, as well as from dental plaque and feces (29-31). There is also one report on *H. pylori* culture from the liver of a patient with Wilson disease (32).

The best culture results are obtained when specimens are inoculated into culture media within 4 hours after collection. If delay is inevitable, the specimen should be transported to the laboratory in a proper transport medium (such as Stuarts transport medium) within 24 hours at 4°C.

**Microscopy of gastric biopsy**

On a Gram stain of smears or imprints of gastric biopsies, *H. pylori* appears as curved, Gram-negative rods. *H. felis* or *H. heilmanii* that can be also present in gastric specimens are usually easily distinguished from *H. pylori* by their long corkcrew shape.

**Culture**

Because *H. pylori* grows slowly and readily transforms into coccoid forms in liquid media, and such cultures are prone to contamination with other fast-growing microorganisms, liquid media are not used for routine primary cultures. Clinical specimens are usually inoculated into solid agar media (such as Columbia or Brucella agar) containing 7-10% of sheep or horse blood and selective antibiotics (eg. Dents or Skirrows selective supplements). The plates are incubated in microaerophilic conditions (2-5% O₂, 5-10% CO₂ and 0-10% H₂) at 37°C for up to 7 days. *H. pylori* grows as small (0.5-2 mm) translucent, yellowish or pale grey colonies. It is identified by positive urease, catalase and oxidase tests. However, these enzymes are also produced by other *Helicobacter* species, and urease can be synthesised by some strains of *Campylobacter lari*.

**Detection of bacterial genetic material**

Various molecular biology methods have been used to detect *H. pylori* DNA or RNA in clinical samples. These methods are usually more sensitive than culture, and are very useful in the evaluation of eradication efficacy. However, except for clarithromycin, they do not allow to determine *H. pylori* antimicrobial susceptibility. Detection of the genetic material of *H. pylori* and other *Helicobacter* species in extragastric tissues, e.g. in the liver, gallbladder or atherosclerotic plaque, stimulated an interest in the possible association between *Helicobacter* infection and other human diseases, such as primary biliary cirrhosis, autoimmune hepatitis or coronary heart disease (33-35).

**H. pylori susceptibility testing and current resistance in Poland**

*H. pylori* treatment includes a combination of an antisecretory drug (usually a proton pump inhibitor) and two or more antimicrobial agents such as
clarithromycin, amoxicillin, metronidazole, or less frequently tetracycline, ciprofloxacin, levofloxacin or moxifloxacin. It has been shown that the *H. pylori* resistance to the antimicrobials used in therapy is one of the most important reasons for eradication failure. Megraud, who analysed many clinical studies, calculated the eradication rates according to the *H. pylori* antimicrobial susceptibility status (*Table 1*) (4). No eradication was achieved when *H. pylori* was simultaneously resistant to clarithromycin and metronidazole and both agents were used in therapy. The lowest eradication rates (18%) were obtained with a triple therapy containing amoxicillin and clarithromycin when *H. pylori* was resistant to clarithromycin. If *H. pylori* was resistant to metronidazole used in a triple therapy together with amoxicillin, successful eradication was observed in 64%. Therefore, it is very important to monitor local resistance, to guide an empirical treatment. Routine susceptibility testing is recommended in environments with a high *H. pylori* resistance, because of the risk of empirical therapy failure and after unsuccessful eradication.

*H. pylori* susceptibility testing is performed by agar dilution or Etest methods. There is a very good correlation between these two methods for clarithromycin, but not for metronidazole (36). More strains are categorised as resistant to metronidazole by Etest than by an agar dilution method. Therefore the latter is recommend as a gold standard for *H. pylori* susceptibility testing. However, agar dilution methods are time- and labour-consuming, and the plates containing antibiotics must be prepared directly before use. Therefore, these methods are rarely used for routine procedures.

A multicentre study on *H. pylori* resistance was conducted in Poland between 2001 and 2004 (*Table 2*) (37). A total of 337 isolates were cultured from adults and children at six centres in the country. All strains were susceptible to amoxicillin and tetracycline. The overall resistance to clarithromycin was 28%, the primary resistance was 22% and the secondary resistance (after previous eradication failure) was 54%. However, the rates of clarithromycin resistance varied substantially between the centres (from 0% to 33%) and between child and

### Table 1. Eradication efficacy according to *H. pylori* susceptibility status (based on ref. 4)

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Resistance to</th>
<th>Eradication rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPI + CL + MTZ</td>
<td>CL</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>MTZ</td>
<td>73%</td>
</tr>
<tr>
<td></td>
<td>CL + MTZ</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No resistance</td>
<td>97%</td>
</tr>
<tr>
<td>PPI + AMX + CL</td>
<td>CL</td>
<td>18%</td>
</tr>
<tr>
<td></td>
<td>No resistance</td>
<td>88%</td>
</tr>
<tr>
<td>PPI + AMX + MTZ</td>
<td>MTZ</td>
<td>64%</td>
</tr>
<tr>
<td></td>
<td>No resistance</td>
<td>89%</td>
</tr>
</tbody>
</table>

PPI, proton pump inhibitor; CL, clarithromycin; AMX, amoxicillin; MTZ, metronidazole
adult isolates (28% versus 15%, respectively). It has been suggested that high clarithromycin resistance in isolates obtained from children can be associated with the frequent use of newer macrolides (such as clarithromycin, roxithromycin or azithromycin) in the treatment of respiratory tract infections in these patients. The overall prevalence of metronidazole resistance was 46%, and the rates of primary and secondary resistance were 41% and 68%, respectively. Metronidazole resistance was significantly higher in strains isolated from women than from men (58.5% versus 37%, respectively), which may possibly be related to the frequent use of nitroimidazoles (metronidazole, tinidazole) in gynaecological infections. As much as 20% of all isolates were simultaneously resistant to clarithromycin and metronidazole.

The results of this study indicate that clarithromycin cannot generally be recommended for empirical eradication therapy in Poland. Its empirical use should be restricted to centers which perform constant susceptibility monitoring and observe low resistance to this agent. Otherwise clarithromycin should be replaced with amoxicillin, which can be combined with metronidazole, since metronidazole resistance seems to be of lesser clinical importance than resistance to clarithromycin.

**Influence of eradication therapy on gastrointestinal microflora and the role of probiotics for the prevention and treatment of *H. pylori* infection**

Although very efficacious (>90%), eradication therapy also has some disadvantages. It can cause side effects (mainly gastrointestinal) which make some patients unable to complete the treatment. Additionally, it leads to qualitative and quantitative disturbances of gastrointestinal microflora (from the oral cavity up to the large intestine), and to increasing resistance both in *H. pylori* and other colonising bacteria. It has been shown that a 7-day treatment with omeprazole, clarithromycin and metronidazole, and to a lesser extent with

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**Table 2. Antimicrobial susceptibility of 337 isolates of *H. pylori* collected in Poland from January 2001 to December 2004 (based on ref. 37)**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Overall</th>
<th>Primary</th>
<th>Secondary</th>
<th>Primary in children</th>
<th>Primary in adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>28</td>
<td>22</td>
<td>54</td>
<td>28</td>
<td>15</td>
</tr>
<tr>
<td>MTZ</td>
<td>46</td>
<td>41</td>
<td>68</td>
<td>40</td>
<td>42</td>
</tr>
<tr>
<td>CL + MTZ</td>
<td>20</td>
<td>13</td>
<td>46</td>
<td>16.5</td>
<td>9</td>
</tr>
<tr>
<td>AMX</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

CL, clarithromycin; MTZ, metronidazole; AMX, amoxicillin; TC, tetracycline
omeprazole, amoxicillin and metronidazole, inhibits the growth of anaerobic bacteria in the oral cavity and the large intestine, selects for resistant streptococci, enterococci and Enterobacteriaceae (especially *Klebsiella* sp.), and increases *Candida* colonisation (38). Although most of these alterations usually normalize within a few weeks after therapy, some of them (such as macrolide-resistant enterococci) may persist for years (39). It is important to remember that even if some patterns of resistance (such as the above-mentioned resistance of enterococci or *Bacteroides* to macrolides) have no direct clinical implications, resistant bacteria can serve as a reservoir of resistance genes for other potential pathogens.

All these limitations of eradication therapy stimulate research on alternative methods of prevention and treatment of *H. pylori* infection. Some promising results have been obtained from *in vitro* and *in vivo* studies with probiotics. It has been shown that some *Lactobacillus* strains inhibit growth or kill *H. pylori in vitro*. These effects can probably be attributed to large amounts of lactate produced during *Lactobacillus* metabolism, or other substances such as microcin (40,41). *L. salivarius* WB 1004 strain inhibits *H. pylori* adhesion to mouse and human gastric epithelial cells, and reduces the secretion of proinflammatory IL-8 (42). Additionally, some *L. reuteri* strains have a surface glycolipid-binding protein similar to *H. pylori*, and therefore can compete with *H. pylori* for receptors on host cells (43). *In vivo* studies revealed that the presence of large amounts of lactobacilli in the stomach prevents *H. pylori* infection in mice challenged with these bacteria, and in previously infected mice a diet containing *L. salivarius* greatly reduces *H. pylori* colonisation density (42). Encouraging results have also been obtained in human studies. In some patients the use of probiotics alone led to eradication or a significant reduction of *H. pylori* colonisation in the stomach (44, 45). Bazzoli *et al.* (46) reported *H. pylori* clearance in 4 out of 20 dyspeptic patients treated with *L. acidophilus* for 8 weeks. In children, dietary products containing live *L. johnsoni* La1 given for 4 weeks significantly decreased *H. pylori* colonisation, as measured by the 13C-urea breath test (47). Several authors have shown that probiotics combined with a standard eradication regimen increased eradication efficacy and/or reduced the side-effects of treatment (48-50). Sykora *et al.* observed significantly higher eradication rates in children treated with omeprazole, clarithromycin and amoxicillin together with fermented milk containing *L. casei* DN-114 001, as compared to patients not receiving probiotic (49). Increased eradication could result from both direct antibacterial activity or stimulation of the immune response by probiotic bacteria. Improved tolerance that is most likely associated with the beneficial influence of probiotics on the gastrointestinal microflora (51) can positively affect patients’ compliance, and therefore increase eradication efficacy.

Some other products, such as melanoidins or culinary and medicinal plants, have also been investigated as alternative options in the treatment of *H. pylori* infections. Melanoidins present in heat-treated food products have been shown to
significantly reduce *H. pylori* colonisation in mice and in humans, as measured by *H. pylori* stool antigen test (52). Several plants, including turmeric, ginger, chilli and parsley killed *H. pylori* and/or suppresed its adhesion to gastric epithelial cells *in vitro* (53). Further studies will probably elucidate if these alternative approaches have a true clinical benefit.

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