Secondary antibiotic resistance and prevalence of Helicobacter pylori  cag PAI genotypes in patients from Croatia

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**Background:** Development of antibiotic resistance due to increased use of antibiotics is one of the major cause for Helicobacter pylori eradication failure. The presence of cagPAI virulence genes, especially cagA gene is associated with severe gastrointestinal diseases.

The aim of the study was to determine antimicrobial susceptibility of H. pylori isolates of symptomatic patients with benign endoscopic finding, after multiple treatmant failures and to detect cagPAI virulence genes.

**Material/methods:** The study comprised 103 patients (78 females and 25 males, mean age 55.8 years and range 28-81 years) with gastric symptoms (abdominal pain, heartburn, vomiting, bloating) after treatman failures. According to the endoscopic findings patients were classified in three groups: non ulcer dyspepsia NUD (n=68), erosio ventriculi EV (n=22), erosio duodeni ED (n=13).

The antibiotic susceptibility testing were determined by E-test for clarithromycin, azithromycin, amoxycillin, metronidazole and levofloxacain. Genes encoding virulence factors were detected by PCR with primers
for 10 loci in cagPAI: Apcag (cagA promotor region), cagA1, cagA2, cagA3, cagM, cagT, cagE, LEC, tnpA and tnpB.

**Results:** Antimicrobial susceptibility testing revealed resistance of 74.8% to azithromycin (AZT) and clarithromycin (CLR), 80.6% to metronidazole (MTZ) and 14.6% resistance to levofloxacin (LEV). There was no resistance to amoxycillin.

Five isolates were susceptible to all tested antibiotics. Resistance to only one antibiotic was found in minor number of isolates and was associated with MZT. The majority of isolates were resistant to more than one antibiotic. Double resistance as noticed for CLR&AZT (n=12), MTZ&LEV (n=4). Triple resistance was found for CLR&AZT&MTZ (n=54), CLR&AZT&LEV (n=3). Resistance to four antibiotics (CLR&AZT&MTZ&LEV) was identified in 8 isolates. There was no statistically significant difference in resistance rates according to the gender (2.5±0.9 compared to 2.4±1.1, t=0.413, P=0.680).

The frequency of single cagPAI genes were as follows: Apcag 63.1%, cagA1 71.8%, cagA2 69.9%, cagA3 5.8%, cagM 71.8%, cagE 75.7%, cagT 68% tnpA 9.7%, tnpB 7.8% and LEC 48.5%. Analysis of resistance rates and presence of particular cagPAI genes revealed no statistically significant correlation (azithromycin, clarithromycin p>0.150, metronidazol p>0.130, levofloxacin p>0.090 for all tested cagPAI genes).

**Conclusions:** Secondary resistance of our *H. pylori* isolates after one or more treatment failures was very high. More than 50% isolates displayed double resistance to macrolides (azithromycin/clarithromycin) and metronidazol while 14.6% to levofloxacin. The study showed high prevalence of cagA, Apcag, cagM, cagE, cagT genes in patients with benign endoscopic findings which could increase the risk of ulcer, pemalignant or malignant disease. Due to these finding it would be necessary to insist on eradication of *H. pylori* infections in order to prevent the development of severe gastroduodenal diseases.

Clinical isolates of *H. pylori* after treatment failures in Croatia demonstrated high rates of antibiotic resistance and high degrees of virulence.