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EVALUATION OF ANTIMICROBIAL RESISTANCE OF *HELICOBACTER PYLORI* IN THE LAST 15 YEARS IN WEST POLAND

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Increasing resistance to drugs represents a serious problem in treatment of infections with *Helicobacter pylori*, providing cause of frequent therapeutic failures. Present study aimed at analysis of changes in resistance of *H. pylori* to antibiotics in West Poland within the recent 15 years. 108 strains of *H. pylori* were analysed, isolated from gastric mucosa of adult patients. Group 1 involved 66 strains isolated in years of 1998/1999. Group 2 comprised 42 isolates obtained in years of 2013/2014. Susceptibility to amoxicillin (AMX), clarithromycin (CL), tetracycline (TC) and metronidazole (MTZ) was determined by E-test (AB Biodisc). All strains on both studied groups were susceptible to AMX. In group 1 all strains proved to be susceptible to TC, while 9% and 36% of tested strains were resistant to CL and MTZ, respectively. By contrast, in group 2, 31% and 83% of strains were resistant to CL and MTZ, respectively. In parallel, 14% strains were found to be resistant to TC (according to EUCAST interpretations). In West Poland, within recent 15 years a dramatic increase was noted in *H. pylori* strains resistant to metronidazole. In parallel, a significant increase was noted in proportion of strains resistant to clarithromycin.

Keywords: *Helicobacter pylori,* antibiotic resistance, chronic gastritis, peptic ulcer disease, treatment

Introduction

Helicobacter pylori represents one of the most widespread pathogenic bacterial species affecting humans. Infections with *H. pylori* are common, burden-

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ing around 50% of world populations [1]. In north European populations about 30% of adults are infected, whereas in south and east Europe the prevalence of *H. pylori* is often higher than 50%. The highest prevalence of *H. pylori* amounting to 84.2% was reported in Portugal [2]. *H. pylori* represents an etiological factor of peptic ulcer disease, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, chronic gastritis with intestinal metaplasia and of gastric adenocarcinoma [3]. Triple therapy including proton-pump inhibitor, amoxicillin and clarithromycin or metronidazole was generally accepted as the first-line therapy [4, 5]. However, the therapy proved to be ineffective in up to 30–40% of patients [6, 7]. An important predictor of the success of *H. pylori* eradication therapy is the antimicrobial susceptibility. In *H. pylori* manifestation of resistance to antibiotics varies geographically and undergoes dynamic alterations [8]. Therefore, several research institutions throughout the world monitor current drug resistance in *H. pylori*. Present study aimed at analysis of changes in resistance of *H. pylori* to antibiotics in West Poland within the recent 15 years.

Materials and Methods

Bacterial isolates

Evaluation of drug susceptibility was performed on the total of 108 strains of *H. pylori* originating from adult patients from West Poland. All the strains were isolated from gastric mucosa before treatment. Group 1 involved 66 strains isolated in years of 1998/1999. Group 2 comprised 42 isolates obtained in years of 2013/2014. Biopsies isolated from the prepyloric portion were immediately placed in a transport medium (Portagerm pylori; bioMerieux). The obtained biopsies were plated on Columbia agar supplemented with 7% of sheep blood and a set of antibiotics (*H. pylori* selective supplement Dent SR 147E; Oxoid). The incubation was performed in microaerophilic conditions (Generbag or Generbox microaer; bioMerieux) at the temperature of 37 °C for 4–7 days. For the drug susceptibility test a suspension of grown bacteria was used, in PBS, manifesting density 2 in McF scale. The cultured strains were identified based on colony morphology, Gram staining and urease and catalase tests. All the research protocols were reviewed and approved by the Ethics Committee of the Poznan University of Medical Sciences, Poland.

Antimicrobial susceptibility testing

Susceptibility to amoxicillin (AMX), clarithromycin (CL), tetracycline (TC) and metronidazole (MTZ) was determined by E-test (AB Biodisc; Solna). A strip was placed on Columbia agar supplemented with 7% of sheep blood with pre-plated, examined strain of *H. pylori*. The incubation was performed in microaerophilic conditions (Generbag or Generbox microaer; bioMerieux) at the temperature of 37 °C for 3 days. Resistance breakpoints of *H. pylori* were used and interpreted according to CLSI (AMX – 0.5 mg/L, CL – 1 mg/L, TC – 4 mg/L, MTZ – 8 mg/L) and according to EUCAST (AMX – 0.12 mg/L, CL – 0.5 mg/L, TC – 1 mg/L, MTZ – 8 mg/L).

Statistical methods

Statistical analysis was performed using Fisher's exact test. A p-value higher than 0.05 was considered non-significant.

Results

All examined *H. pylori* strains in both groups were susceptible to amoxicillin (AMX). Group 1 contained no strains resistant to tetracycline (TC), in turn the group comprised 9% of strains resistant to clarithromycin (CL) and 36% of strains resistant to metronidazole (MTZ). On the other hand, Group 2 contained 31% of strains resistant to CL and 83% of strains resistant to MTZ. Resistance to TC on Group 2 depended on the applied criteria: according to EUCAST it involved 14% but none after CLSI recommendations. In line with the currently binding criteria of EUCAST, a significant increase in their content of strains resistant to TC ($p < 0.0001$), CL ($p = 0.0003$) and MTZ ($p < 0.0001$) was detected between the two groups. The results obtained in both groups of studied strains are presented in Table I.

Discussion

In treatment of infections with *H. pylori*, the universal and still applied triple therapy is also defined as first-line therapy [5]. At present several factors are known which may reduce efficacy of the standard triple therapy: they include the high bacterial load, type of strain, high gastric acidity and increase in *H. pylori* resistance to antibiotics [9].

Table I. Resistance of *Helicobacter pylori* to AMX, CL, TC and MTZ in Group 1 (years 1998/1999) and Group 2 (years 2013/2014) according to EUCAST breakpoints

Antibacterial drug	Percent of resistance in studied groups of *H. pylori* strains		Level of significance; p
	Group 1 (1998/1999)	Group 2 (2013/2014)	
AMX	0%	0%	1.0000
CL	9%	31%	0.0003*
TC	0%	14%	<0.0001*
MTZ	36%	83%	<0.0001*

*significantly different between both groups.

In the conducted experiments none of the analysed strains manifested resistance to AMX. Nevertheless, in recent decade efficacy of eradication in *H. pylori* infection following the triple therapy was found to be reduced [10]. In such a context it remains difficult to interpret the demonstrated susceptibility of isolates to AMX in both studied groups. Possibly, the clinical resistance of *H. pylori* to AMX depends on bioaccessibility of the drug in gastric mucosa linked to its acidity [9]. Our results have been confirmed by other European data, documenting the level of resistance to amoxicillin of 0–0.9% [11]. A high proportion of primary amoxicillin resistance (13.6–59%) was detected in Africa, Asia and South America [12–14].

In the studies presented above the proportion of strains resistant to clarithromycin in West Poland was found to comprise at present 31% and it increased by 22% within recent 15 years. In addition, recent data indicate that genotype studies (by PCR) allow for a markedly more frequent detection of resistance to clarithromycin than using phenotypic determination (by E-test) [15]. In Europe clarithromycin resistance manifests a variable level and in strains isolated from adult patients in 2008–2009 on averaged at 17.5%. The highest proportion of strains resistant to clarithromycin was detected in Greece (42%) and the lowest one in Holland (5.6%) [11, 16]. In Poland, resistance to clarithromycin was gradually increasing, amounting in 1998/1999 to 9% [17], in 2001–2009 already to 15.4–28% [18–20]. In studies presented here we have demonstrated 31% resistance of *H. pylori* strains to clarithromycin, which might point to a continuous increase in resistance to the drug. The increase in resistance to clarithromycin was observed also in strains isolated from children, linked to the widespread application of macrolide antibiotics in paediatrics [19, 21, 22]. Therefore, clarithromycin should not be applied in treatment of *H. pylori* infections without earlier estimation of susceptibility to the drug.

In the conducted studies a significant increase was noted in proportion of strains resistant to TC (0% in Group 1 versus 14% in Group 2). However, the data were obtained using interpretation criteria of EUCAST. On the other hand, using criteria of CLSI, no strain in either of the two groups manifested resistance to TC. Using CLSI criteria, other authors [11, 23, 24] demonstrated in Europe persistence of low resistance to TC (0–2.6%). Since interpretation criteria of EUCAST related to resistance of *H. pylori* to TC may result in divergent results, as compared to those obtained in line with the earlier CLSI recommendations, the matter requires verification.

In this study we have for the first time demonstrated a dramatic increase in resistance to metronidazole (83%) among strains isolated in 2013/2014. A similarly high frequency (50–80%) of strains resistant to the chemotherapeutic agent was noted in developing countries [12]. Between 2000–2010 in various European countries 20–43.8% of strains were found to be resistant to metronidazole. The highest proportion of resistant strains was demonstrated in Italy (59.3%) and in countries of Central and Western Europe (>40%) [11, 25]. In Poland, in 1998/1999 resistance to metronidazole was manifested by 36% strains [17] and the proportion systematically grew in subsequent years, including 41.7–58.5% in 2000–2004 [19, 20], and up to 66.7% in certain southern regions of Poland in 2008–2011 [26]. It seems probable that the significant increase in resistance to metronidazole in Poland reflects a sequel of using the drug not only in treatment of gynaecological and dental diseases but also as a drug for eradication of parasitic infections [22].

Thus, in West Poland, within recent 15 years a dramatic increase was noted in *H. pylori* strains resistant to metronidazole as well as in percentage of strains resistant to clarithromycin. In parallel, the EUCAST criteria for interpretation of *H. pylori* resistance to tetracycline require verification.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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