Biotechnology. Theory and Practice/Биотехнология. Теория и практика.
2015, no. 4, pp. 4-9
DOI: 10.11134/btp.4.2015.1

УДК 616.36-092:579.835.12

ANTIBIOTIC RESISTANCE OF *HELICOBACTER PYLORI* ISOLATES FROM KAZAKH PATIENTS

Kulmambetova G.N.[2], Bekenova E.E.[1], Tujakova A.K.[1], Kozhakhmetov S.S.[1], Logvinenko A.A.[3], Sukashev A.T.[3], Almagambetov K.K.[1]

[1]Republic Collection of Microorganisms
13/1, Valikhanov str., Astana, 010000, Kazahstan
[2]National Center for Biotechnology
13/5, Korgalzhyn road, Astana, 010000, Kazakhstan
[3]National Research Medical Center
42, Abylai Khan ave., Astana, 010000, Kazakhstan
gulmirakn@gmail.com

ABSTRACT

***Helicobacter pylori* strains can be resistant to important triple combination therapies for *H. pylori* eradication. The aim of this study was to investigate the rate of resistance to clarithromycin, metronidazole, amoxicillin, tetracycline, and rifampicin, as well as to detect antibiotic resistance-associated mutations in *H. pylori* isolates from patients in Kazakhstan. Susceptibility of 20 *H. pylori* strains was tested using the E test method. Genes associated with resistance and susceptible clinical isolates were sequenced in order to assess resistance and non-resistance associated genetic alterations. Of the 20 clinical isolates examined, 8 (40%) showed phenotypic resistance to metronidazole (MIC > 256 mg/L), 13 (65%) to clarithromycin (MIC > 256 mg/L), and 1 (5%) to amoxicillin (MIC > 6 mg/L). The majority of resistant strains had point mutations in the 23S rRNA gene and rdxA gene, and one strain had mutations in the pbp1A gene. The remaining isolates with moderate resistant isolates (MIC <0.016 mg/L) demonstrated a drug-susceptible phenotype, and did not harbour any mutation in the gene sequences evaluated. In the Kazakh population of existing clarithromycin and metronidazole resistance, tetracycline, amoxicillin, and rifampicin could prove useful for rescue regimens in patients with previously unsuccessful *H. pylori* eradication regimens.**

Keywords: *Helicobacter pylori*, clarithromycin, metronidazole, amoxicillin, tetracycline, rifampicin

INTRODUCTION

Helicobacter pylori is one of the most common worldwide infection, that induces stomach complications such as gastritis, peptic ulcer, adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma [1]. *Helicobacter pylori* was detected in 50% of the world population [2, 3]. In medical practice has recommended to eradicate *Helicobacter pylori* in symptomatic patients. For treatment commonly use of triple agent therapy with a double-dose proton pump inhibitor (PPI) and two antibiotics chosen from amoxicillin, clarithromycin and metronidazole, thereby eradication rate is from 70-80% [4]. Today, we have increasing problem with antibiotic resistance for the treatment of infectious diseases. In *Helicobacter pylori* have been investigated several genetic determinants for resistance to several antibiotics, including clarithromycin, metronidazole, amoxicillin, tetracycline and rifampicin [5]. Clarithromycin is associated with mutations in the 23S rRNA gene, which inhibit the binding of drug to the ribosome [6]. Metronidazole resistance may have different mechanisms, but the dominant determinant has been shown mutations in the rdxA gene that encodes an oxygen-insensitive NADPH nitroreductase [2]. Bacterial resistance against β-lactam antibiotics such as amoxicillin have been determined in structural alterations in one of the penicillin-binding proteins (pbp1A) or changes in other proteins involved in cell wall synthesis [7]. Tetracycline has been binded with the ribosome, prevents connection to the aminoacyl-tRNA and the subsequent synthesis of proteins [8]. Rifampicin resistance exists from mutations in the rpoB gene, encoding the β subunit of RNA polymerase [9].

Data about *Helicobacter pylori* resistance to antibiotics don't exist in Kazakhstan. The aim of our work was to investigate the resistance rate to clarithromycin, metronidazole, amoxicillin, tetracycline and rifampicin and the association of mutations in 20 strains *Helicobacter pylori* from symptomatic Kazakh patients.

MATERIALS AND METHODS

Study population

A total of 20 *H. pylori* strains were isolated between 2012 and 2013 from 20 symptomatic patients from National Research Medical Center (Astana, Kazakhstan). Median age was 36.2 years (range, 18-57 years), and 55% of the patients (n=11) were males. The predominant ethnic group were Kazakhs (80%), and Russian (20%),

in keeping with the ethnic distribution in Kazakhstan. On the basis of endoscopic findings, 9 had gastritis, 7 had ulcerated lesions, and 4 had duodenal ulcer.

No patient had previously received anti-*H. pylori* therapy. Biopsy sample was cultured for *H. pylori* isolation. Written informed consent was obtained from all of the patients and the study protocol was approved by the ethics committee of the National Center for Biotechnology.

H. pylori strains, growth conditions, and antibiotics

H. pylori culture was grown using Columbia agar plates with 20% defibrinated horse blood, 10% yeast extract and *H. pylori* selective antibiotic supplement (Oxoid, Basingstoke, UK) colistin (10mg/L). The plates were incubated for up to 7 days at 37^0C in microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2). *H. pylori* was identified by colony and microscopic morphology, and by genotyping 16S rRNA gene.

Determination of MIC

The MIC was routinely determined by the E-test method (Biomerieux, France). The classification of *H. pylori* as resistant strains were performed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint tables for interpretation of MICs and zone diameters (http://www.eucast.org/clinical_breakpoints/).

DNA extraction and PCR amplification

H. pylori genomic DNA was extracted with the Wizard® Genomic DNA purification kit (Promega, USA). DNA purified with this system is suitable for an amplification.

The fragments of the genes were amplified by PCR. All primers have shown in Table 1. The PCR was carried out in 20 µL reaction solution containing 1 nM DNA of *H. pylori* genomic DNA, 250 nM of each primer, 10x PCR buffer, 2.5 uM $MgCl_2$, 200 nM of each dNTP, 1U of DNA polymerase. Amplification was performed under the following conditions: 5 minutes at 95^oC to initiate denaturation, further 30 cycles of 30 sec at 95^oC, 30 sec at 55^0C for rdxA and pbp1A genes, and 59^oC for 23S, 16STCR and rpoB, 40 sec at 72^oC by using GeneAmp® PCR System 9700 (Applied Biosystems, USA). PCR products were sequenced on ABI 3730xl automatic genetic analyzer (Applied Biosystems, USA). The obtained data were analyzed with SeqScape v 2.6 software (Applied Biosystems, USA).

Table 1. PCR primer pairs used to amplify portion of *H. pylori* genes

Таблица 1. Праймеры, используемые при амплификации генов *H. Pylori*

Gene	Nucleotide sequence ($5' \rightarrow 3'$)	Size (bp) of PCR fragment
23S rRNA	CGTAACTATAACGGTCCTAAG TTAGCTAACAGAAACATCAAG	291
rdxA	AGGGATTTTATTGTATGCTACAA AGGAGCATCAGATAGTTCTGA	886
pbp1A	GCTATTCCACGACTTCTAAA GCAAGGTTACAAGCCCTAAA	135
16STCR	GGTAGTCCACGCCCTAAACGA GGGTTGCGCTCGTTGCGGGA	322
rpoB	TTTGATTCGCTCATGCCCCAT CACAACCTTTTTATAAGGGGC	330

Statistical analysis

The chi-square test was used to compare rates of resistance to antibiotics during the study period. P value of ≤ 0.05 were considered statistically significant. All analyses were performed by using R program.

RESULTS

Antimicrobials susceptibility and mutational analysis antibiotics resistance of _H. pylori_

Twenty *H. pylori* isolates were cultured from gastric biopsy samples obtained from patients during endoscopy at National Research Medical Center (Astana) between 2012 and 2013.

Biotechnology. Theory and Practice/Биотехнология. Теория и практика.
2015, no. 4, pp. 4-9
DOI: 10.11134/btp.4.2015.1

According E-test, all isolates were characterized by no resistance to rifampicin (MIC range, <0.016-0.125 mg/L with R>1 mg/L), and tetracycline (MIC range, <0.016-0.75 mg/L with R>1 mg/L). Amoxicillin has been determined one resistance strain (MIC range, <6 mg/L with R>0.125 mg/L), clarithromycin (MIC range, <0.016-≤0.25 mg/L with S≤0.25 mg/L), except for some clarithromycin-resistant strain (MIC range, >256 mg/L with R>0.5 mg/L). The isolates were characterized by susceptible rates of resistance to clarithromycin (MIC range, ≤4 mg/L with R>0.5 mg/L) (65%, n=13) and high rates of resistant to metronidazole (MIC range, ≥32 mg/L with R>8 mg/L) (40%, n=8) (table 2, 3).

Table 2. Resistance to antibiotics of *H. pylori* isolates in Kazakhstan from 2012 to 2013

Таблица 2. Резистентность *H. pylori* к антибиотикам в Казахстане с 2012 по 2013

Antibiotic	Number of resistant strains (%)	MIC (mg/L)	
		Median	Range
Amoxicillin	1 (5)	<0.38	<0.016->6
Tetracycline	0	0.4	<0.016-0.75
Rifampicin	0	<0.04	<0.016-0.125
Metronidazole	8 (40)	77	<0.016->256
Clarithromycin	13 (65)	14	<0.016->256

Mutations and MIC values observed in 20 strains are summarized in table 3. Eight strains had 2 or more mutations with the highest number of mutations. In this study mutation T2182C, associated with resistance to clarithromycin, was the most prevalent mutation while mutations A2142G and A2143G were detected in few strains. Ten of the 20 strains possessed T2182C mutation (table 4). The frequency of A2143G mutation was very low in one strain-3 of 20 strains (5%). The MIC of clarithromycin for these mutants (A2142G and T2182C) ranged from 0.016 to 256 mg/L, the strain considered resistant if MIC value of strain exceeded >0.5 mg/L and susceptible if MIC value of strain is under ≤0.25 mg/L. Of the 8 strains found resistance to metronidazole. All of them were found to have a mutation in the rdxA gene (table 3). One of them has a C-to-T mutation that leads to the amino acid change of Ala67 to Val. A single base substitution mutation (C to T) was also found in another mutant that results a transversion of Glu175 stop. The other six mutants were due to insertion of an adenine in a run of six adenines, causing the shift of the reading frame (at position 9) that encounters a stop codon at position 23 (truncation of the protein). Two of the 8 strains had the deletion of a G, that also occurred to the shift of the reading frame (at position 133) in a stop codon at position 137 (protein). The MICs for the Mtz (Metronidazole) mutants varied, ranging from 0.016 to 256 μg/mL (table 3). The 1 strain had resistance to amoxicillin with amino acid change mutation of Ser_{414} – Arg, that indicated the main factor of amoxicillin resistance than Glu_{406} – Ala (table 3).

Table 3. Mutations and MIC value associated with resistance to clarithromycin, metronidazole and amoxicillin

Таблица 3. Мутации и значения МПК, ассоциированные с резистентностью к кларитромицину, метронидазолу и амоксициллину

Strain	Designation	Mutant alleles Amino acid change	MIC breakpoint mg/L				
			Amoxicillin S≤0.125 R>0.125	Clarithromycin S≤0.25 R>0.5	Metronidazole S≤8 R>8	Tetracycline S≤1 R>1	Rifampicin S≤1 R>1
1	23S pbp1A pbp1A	A2142G Glu_{406} – Ala Ser_{414} – Arg	6	1.5	1.5	0.75	0.064
2	23S rdxA rdxA	T2182C -G G→T E175st	0.047	2	256	0.50	0.064
3	23S rdxA	A2143G +A	0.032	0.75	96	0.25	0.094
4	23S	T2182C	0.032	1.5	2	0.125	0.023
5	23S rdxA	A2142G C→T A67V	0.064	1.5	256	0.25	0.016
6	-	-	0.064	0.25	0.75	0.75	0.032

7	23S rdxA	T2182C +A	0.023	0.75	64	0.19	0.047
8	23S	T2182C	0.094	0.75	8	0.50	0.016
9	23S	T2182C	0.047	1	6	0.75	0.125
10	23S	T2182C	0.094	0.75	4	0.064	0.094
11	23S rdxA	T2182C +A	0.016	256	128	0.38	0.023
12	23S rdxA	T2182C +A	0.023	1.5	256	0.19	0.047
13	23S rdxA rdxA	T2182C +A -G	0.0125	1	256	0.25	0.016
14	-	-	0.094	0.064	2	0.75	0.023
15	pbp1A	Glu_{406} – Ala	0.064	0.016	2	0.75	0.047
16	pbp1A	Glu_{406} – Ala	0.023	0.19	3	0.125	0.03
17	pbp1A	Glu_{406} – Ala	0.125	0.125	1	0.19	0.025
18	rdxA	+A	0.032	0.016	192	0.094	0.094
19	23S	T2182C	0.064	1	1.5	0.38	0.023
20	-	-	0.25	0.023	3	0.25	0.064

Table 4. Distribution of mutations amongst 20 strains

Таблица 4. Распределение мутаций среди 20 штаммов

Mutations	Total number of strains	% of strains with each mutation type
A2142C	0	0
A2142G	2	10 (2/20)
A2143G	1	5 (1/20)
T2182C	10	50 (10/20)
AGA→TTC	0	0
GGTGC	0	0
Shift at 133, stop at 137	2	10 (2/20)
E175 stop	1	5 (1/20)
Shift at 9, stop at 23	6	30 (6/20)
A67V	1	50 (1/20)
Glu_{406}→Ala	4	20 (4/20)
Ser_{41}→Arg	1	5 (1/20)
Total number of mutation types	28	

DISCUSSION

Clarithromycin, metronidazole, amoxicillin, tetracycline and rifampicin the drugs which employed for triple combination therapy in the treatment of *H. pylori* infection. Resistance to the drugs presents a challenge. Different studies all over the world have reported resistance to these drugs [10-12]. Thirteen independent Cla^r (Clarithromycin) mutants were obtained and analysed. The sequencing results showed that two of them are due to the mutation A2142G, one are due to A2143G and the other ten are due to T2182C with rates of resistance (R>0.5 mg/L) (Table 4). These three types of mutations are predominantly associated with Cla^r in clinical isolates, these results also were confirmed with another observations [10]. Clarithromycin is used in the world as potent antibiotic for eradication *H. pylori* and resistance to clarithromycin has been increasingly reported in several studies [13, 14]. Clarithromycin acts by inhibiting protein synthesis by binding to the peptidyltransferase loop of 23S rRNA which has been shown at residues A2058 and A2059 in the 23S rRNA gene *E. coli*. If mutation occurs in these residues, the binding affinity of clarithromycin to ribosomes is reduced, resulting in clarithromycin resistance. Clarithromycin resistance is increasing in most countries in Central, Western and Southern Europe, as well as East Asia, and reached more than 20% [15]. The mutation at A2143G in the 23S rRNA is frequently found in several countries such as Spain 79,4%, Brazil 74%, and Tunisia 88% [16]. Mutation at T2182C was found in 5.9% of isolates in Spain [17]. In Kazakhstan mutation at A2142G and A2143G were 10% and 5% respectively (Table 4). The mutation at T2182C was 50% in *H. pylori* of Kazakh isolates.

Eight independent Mtz mutants were obtained and analysed. Metronidazole resistance in the world ranges 20-40% in Europe and the USA, to 50-80% in developing countries, Iran, India and Egypt showed the highest rates of resistance (80-100%) [18, 19]. Goodwin et al. demonstrated that the loss of oxygen-insensitive NADPH

nitroreductase activity resulted in the development of resistance to metronidazole in *H. pylori* [20]. Several observations demonstrated similar results where the mutations linked with the resistance of metronidazole. In our study the sequence results showed that eight of them had frameshift in different localization and the other 2 had amino acid change with high rates of susceptibility to metronidazole (S≤8 mg/L) (table 4). Changes in the rdxA gene were due to frameshift rather than missense mutations. All of the frameshift mutations resulted in a translational stop codon and hence a truncated rdxA protein. Missense mutations were less common. The amino acid substitution was observed in only two isolates (position 67 and 175). Two isolates had multiple mutations in the rdxA gene. Strain 2 and 13 had two frameshift (table 3). In our study prevalence of the resistance in *H. pylori* of Kazakh isolates showed 40%.

Early studies of amoxicillin resistance were suggested to result from alterations in pbp1A gene [21]. And after some investigation has indicated the Ser414-Arg substitution in pbp1A represents the main factor in amoxicillin resistance that increased the MIC of amoxicillin [7]. Our results indicated that 5% of strains were resistant to the amoxicillin with MIC exceeding >0.125 mg/L, while 6 mg/L was found to be maximum MIC value.

Tetracycline is a protein synthesis inhibitor, active against gram-negative and gram-positive bacteria [22]. Tetracycline binds to the 30S subunit of the ribosome and blocks the binding of aminoacyl-tRNA, thus stalling the synthesis of nascent peptide chains [22]. Tetracycline resistance in *H. pylori* is relatively rare, the majority of studies reporting no tetracycline resistance in clinical isolates [23]. However, with use of tetracycline in therapy, tetracycline-resistant isolates have started to appear at low levels, 5 to 7% of isolates [24]. The clinical isolates from Shanghai, China, the level of tetracycline resistance was 59%. The study from Canada demonstrated that the mutations in the 16S rRNA are clearly responsible for tetracycline resistance in *H. pylori* [22]. Among the Kazakh isolates were 0% of mutation in 16S rRNA gene with MIC range <0.016-0.75 mg/L with R>1 mg/L.

Also we observed the lack of resistance to rifampicin in this study with range of MIC <0.016-0.047 with R>1 mg/L and completely lack of mutations in rpoB gene of *H. pylori* strains. Rifampicin resistance described in Germany, where one clinical isolate has been acquired rpoB gene mutation during therapy [9] Rifampicin can be used in triple therapy as a rescue regimen with unsuccessful *H. pylori* eradication [25]

In summary, high-level drug resistance *H. pylori* in Kazakh population is linked to a point mutation on 23S rRNA and rdxA gene. Amoxicillin, tetracycline, and rifampicin resistance rates were significantly lower than clarithromycin and metronidazole. MIC of rifampicin was much lower than those classical antibiotics used for *H. pylori* eradication. According our results, in Kazakh population of existing clarithromycin and metronidazole resistance, three amoxicillin, tetracycline, and rifampicin could be helpful in rescue regimens with unsuccessful *H. pylori* eradication.

Funding

This study was funded by the Ministry of Education and Science of the Republic of Kazakhstan (grant 0112RK00458). The authors have no conflicts of interest.

REFERENCES

1. Sanders M.K., Peura D.A. Helicobacter pylori-Associated Diseases. *Curr Gastroenterol Rep*, 2002, vol. 4, no. 6, pp. 448-454.

2. Kim S.Y., Joo Y.M., Lee H.S. et al. Genetic analysis of Helicobacter pylori clinical isolates suggests resistance to metronidazole can occur without the loss of functional rdxA. *J Antibiot (Tokyo)*, 2009, vol. 62, no. 1, pp. 43-50. doi: 10.1038/ja.2008.6.

3. Kao C.Y., Lee A.Y., Huang A.H. et al. Heteroresistance of Helicobacter pylori from the same patient prior to antibiotic treatment. *Infect Genet Evol*, 2014, vol. 23, pp. 196-202. doi: 10.1016/j.meegid.2014.02.009.

4. Zhou L., Zhang J., Chen M. et al. A comparative study of sequential therapy and standard triple therapy for Helicobacter pylori infection: a randomized multicenter trial. *Am J Gastroenterol*, 2014, vol. 109, no. 4, pp. 535-541. doi: 10.1038/ajg.2014.26.

5. Secka O., Berg D.E., Antonio M. et al. Antimicrobial susceptibility and resistance patterns among Helicobacter pylori strains from The Gambia, West Africa. *Antimicrob Agents Chemother*, 2013, vol. 57, no. 3, pp. 1231-1237. doi: 10.1186/12-3.

6. Alfaresi M.S., Elkoush A.A. Characterization of clarithromycin resistance in isolates of Helicobacter pylori from the UAE. *Indian J Gastroenterol*. 2010, vol. 29, no. 3, pp. 116-120. doi: 10.1007/s12664-010-0034-z.

7. Gerrits M.M., Schuijffel D., van Zwet A.A. et al. Alterations in penicillin-binding protein 1A confer resistance to beta-lactam antibiotics in Helicobacter pylori. *Antimicrob Agents Chemother*, 2002, vol. 46, no. 7, pp. 2229-2233.

8. Wu J.Y., Kim J.J., Reddy R. et al. Tetracycline-resistant clinical Helicobacter pylori isolates with and without mutations in 16S rRNA-encoding genes. *Antimicrob Agents Chemother*, 2005, vol. 49, no. 2, pp. 578-583.

9. Heep M., Rieger U., Beck D. et al. Mutations in the beginning of the rpoB gene can induce resistance to rifamycins in both Helicobacter pylori and Mycobacterium tuberculosis. *Antimicrob Agents Chemother*, 2000, vol. 44, no. 4, pp. 1075-1077.

10. Kim J.M., Kim J.S., Kim N. et al. Gene mutations of 23S rRNA associated with clarithromycin resistance in Helicobacter pylori strains isolated from Korean patients. *J Microbiol Biotechnol*, 2008, vol. 18, no. 9, pp. 1584-1589.

11. Matteo M.J., Perez C.V., Domingo M.R. et al. DNA sequence analysis of rdxA and frxA from paired metronidazole-sensitive and -resistant Helicobacter pylori isolates obtained from patients with heteroresistance. *Int J Antimicrob Agents*, 2006, vol. 27, no. 2, pp. 152-158.

12. Gerrits M.M., de Zoete M.R., Arents N.L. et al. 16S rRNA mutation-mediated tetracycline resistance in Helicobacter pylori. *Antimicrob Agents Chemother*, 2002, vol. 46, no. 9, pp. 2996-3000.

13. Keshavarz Azizi Raftar S., Moniri R., Saffari M. et al. The Helicobacter pylori Resistance Rate to Clarithromycin in Iran. *Microb Drug Resist*, 2014, vol. 21, doi: 10.1089/mdr.2014.0104.

14. Giorgio F., Principi M., De Francesco V. et al. Primary clarithromycin resistance to Helicobacter pylori: Is this the main reason for triple therapy failure? *World J Gastrointest Pathophysiol*, 2013, vol. 4, no. 3, pp. 43-46. doi: 10.4291/wjgp.v4.i3.43.

15. Seck A., Burucoa C., Dia D. et al. Primary antibiotic resistance and associated mechanisms in Helicobacter pylori isolates from Senegalese patients. *Ann Clin Microbiol Antimicrob*, 2013, vol. 12, pp. 3. doi: 10.1186/12-3.

16. Sakinc T., Baars B., Wuppenhorst N. et al. Influence of a 23S ribosomal RNA mutation in Helicobacter pylori strains on the in vitro synergistic effect of clarithromycin and amoxicillin. *BMC Res Notes*, 2012, vol. 5, pp. 603. doi: 10.1186/5-603.

17. Agudo S., Perez-Perez G., Alarcon T. et al. Rapid detection of clarithromycin resistant Helicobacter pylori strains in Spanish patients by polymerase chain reaction-restriction fragment length polymorphism. *Rev Esp Quimioter*, 2011, vol. 24, no. 1, pp. 32-36.

18. Boyanova L., Mitov I. Geographic map and evolution of primary Helicobacter pylori resistance to antibacterial agents. *Expert Rev Anti Infect Ther*., 2010, vol. 8, no. 1, pp. 59-70. doi: 10.1586/eri.09.113.

19. Sherif M., Mohran Z., Fathy H. et al. Universal high-level primary metronidazole resistance in Helicobacter pylori isolated from children in Egypt. *J Clin Microbiol*, 2004, vol. 42, no. 10, pp. 4832-4834.

20. Goodwin A., Kersulyte D., Sisson G. et al. Metronidazole resistance in Helicobacter pylori is due to null mutations in a gene (rdxA) that encodes an oxygen-insensitive NADPH nitroreductase. *Mol Microbiol*., 1998, vol. 28, no. 2, pp. 383-393.

21. Kwon D.H., Dore M.P., Kim J.J. et al. High-level beta-lactam resistance associated with acquired multidrug resistance in Helicobacter pylori. *Antimicrob Agents Chemother*, 2003, vol. 47, no. 7, pp. 2169-2178.

22. Trieber C.A., Taylor D.E. Mutations in the 16S rRNA genes of Helicobacter pylori mediate resistance to tetracycline. *J Bacteriol*., 2002, vol. 184, no. 8, pp. 2131-2140.

23. Zhang Y., Gao W., Cheng H. et al. Tetracycline- and Furazolidone-containing Quadruple Regimen as Rescue Treatment for Helicobacter pylori Infection: A Single Center Retrospective Study. *Helicobacter*., 2014, vol. 12, no. 5, pp. 382 – 386. doi: 10.1111/hel.12143.

24. Kwon D.H., Kim J.J., Lee M. et al. Isolation and characterization of tetracycline-resistant clinical isolates of Helicobacter pylori. *Antimicrob Agents Chemother*., 2000, vol. 44, no. 11, pp. 3203-3205.

25. Gisbert J.P., Calvet X. Review article: rifabutin in the treatment of refractory Helicobacter pylori infection. *Aliment Pharmacol Ther*., 2012, vol. 35, no. 2, pp. 209-221. doi: 10.1111/j.1365-2036.2011.04937.x.

ҚАЗАҚСТАН ПАЦИЕНТЕРДІҢ *HELICOBACTER PYLORI* ИЗОЛЯТТАРЫНЫҢ АНТИБИОТИК ТӨЗІМІ

Құлмамбетова Г.Н.[2], Бекенова Е.Е.[1], Тұякова А.К.[1], Қожахметов С.С.[1], Логвиненко А.А.[3], Сукашев А.Т.[3], Алмағамбетов Қ.Х.[1]

[1]Микроорганизмдердің республикалық коллекциясы
Уәлиханов к-сі, 13/1, Астана, 010000, Қазақстан
[2] Ұлттық биотехнология орталығы
Қорғалжын тас жолы 13/5, Астана, 010000, Қазақстан
[3] Ұлттық ғылыми медициналық орталығы
Абылайхан данғылы, 42, Астана, 010000, Қазақстан
gulmirakn@gmail.com

ТҮЙІН

***Helicobacter pylori* штаммдарын жою кезінде олар үш компонетті емге төзімділік танытуы мүмкін. Зерттеудің мақсаты кларитромицин, метронидазол, амоксициллин, тетрациклин, рифампицинге төзімділік деңгейін зерттеу, сонымен қатар, Қазақстан науқастарынан бөлініп**

Biotechnology. Theory and Practice/Биотехнология. Теория и практика.
2015, no. 4, pp. 4-9
DOI: 10.11134/btp.4.2015.1

алынған *H. pylori* антибиотикке төзімділігіне байланысты мутацияларды анықтау болып табылады. H. pylori 20 штамының антибиотиктерге сезімталдығы Е тест арқылы тексерілді. Клиникалық изоляттардың төзімділігі мен сезімталдығына жауапты гендер мутациялардың төзімділік пен сезімталдыққа қатысын бағалау үшін секвенделді. Талданған 20 клиникалық изоляттың ішінен 8(40%) метронидазолға (MIC >256 mg/L), 13(65%) кларитромицинге (MIC >256 mg/L), және 1(5%) амоксициллинге (MIC >6 mg/L) фенотиптік төзімділік танытты. Төзімді штаммдардың көбісі 23S рРНҚ, rdxA гендерінде, және бір штамм pbp1A генінде мутация бар екенін көрсетті. Қалған орташа төзімді изоляттар (MIC <0.016 mg/L) дәріге сезімталдық фенотипін және гендер тізбегінде мутация жоқ екенін көрсетті. Қазақ популяциясындағы *H. pylori* штаммдарында кларитромицин мен метронидазолға төзімділік бар. *H. pylori* жою сәтсіз болған жағдайда, тетрациклин, амоксициллин және рифампициннің пайдасы тию мүмкін.

Негізгі сөздер: *Helicobacter pylori,* кларитромицин, метронидазол, амоксицилин, тетрациклин, рифампицин