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Comparative Multiple-Locus Variable-Number Tandem Repeat Analysis of *Helicobacter Pylori* Isolates from South of Russia

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Abstract: High genetic diversity and rapid microevolution are a peculiarity of genomes *Helicobacter pylori*, a phenomenon that is proposed to play a functional role in persistence and colonisation of diverse human populations. Isolates of *H. pylori* can be classified phylogeographically. To characterise diverse strains of this pathogen in different human populations, we compare MLVA genotypes of 48 *H. pylori* strains isolated from different regions in the South of Russia. Cluster analysis identified 48 individual MLVA types (MTs) and revealed the possible belonging the greatest part of the strains to hpEurope, and two strains to hpEastAsia population, when compared to the 4 publically available genomes. The proposed method may be successfully used in molecular epidemiology and possibly for population identification of *H. pylori*. A similar study was conducted in Russia and Europe for the first time.

Keywords: *Helicobacter Pylori,* VNTR Genotyping, MLVA, Cluster Analysis, Molecular Epidemiology

1. Introduction

H. pylori isolates obtained from different individuals and ethnic groups in the world exhibit substantial genomic diversity due to synonymous substitutions, insertion-deletion (indel) polymorphisms, and mobility of repetitive elements. This diversity could be further enhanced by chromosomal rearrangements due to a high level of interstrain recombination. Geographical partitioning of the gene pool exists within *H. pylori*, and sequences are less related between isolates from different populations than between isolates from families [1]. Several molecular typing tools were tried for strain typing and identification of *H. pylori* isolates. These include pulsed-field gel electrophoresis [2], random fragment length polymorphism [3], randomly amplified polymorphic DNA [4, 5], amplified fragment length polymorphism [6, 7], and PCR-based genotyping of repetitive sequences, namely, repetitive extragenic palindromes [8, 9] and enterobacterial repetitive intergenic consensus elements [10]. Phylogenetic analysis based on multi-locus sequence typing (MLST) of several genes revealed geographical differentiation since *H. pylori* left Africa together with Homo sapiens [11]. All these techniques indicate that the *H. pylori* population genetic structure is panmictic, and a high level of DNA diversity is found within strains. However, all of these methods suffer from one or more significant drawbacks, including insufficient discriminatory power, poor reproducibility between laboratories, and difficulties with the comparison and accumulation of results by different laboratories, or application of expensive sophisticated equipment such as DNA sequencers. As an alternative to the above methods, investigation of Variable Number of Tandem Repeats (VNTR) has been described for various organisms. VNTRs can provide information relating to both the evolutionary and functional areas of bacterial diversity [12]. The ability to detect VNTRs

in microorganisms has been greatly enhanced by the availability of whole genomic sequences and software that can search for VNTR loci from these sequences [13]. Furthermore when VNTR is applied to multiple loci as a typing scheme such as in Multiple Locus VNTR Analysis (MLVA) greater discriminatory power and more accurate determination of genetic relatedness is achieved [14, 15]. However, there's little information about application of MLVA typing for *H. pylori* populations [16, 17]. In this paper, new 4 VNTR loci of *H. pylori* genome were identified and used to analyze 48 strains of *H. pylori* which originated from different regions of Russia (Astrahan' and Rostov region).

2. Material and Methods

2.1. Bacterial Strains and DNA Isolation

Twenty-three *H. pylori* isolates were recovered from antral gastric biopsies of patients from Rostov region. For culture, biopsy samples were homogenized and inoculated onto Trypticase soy agar plates supplemented with 7.5% sheep blood. Cultures were identified by urease, catalase, and oxidase tests and Gram staining. Genomic DNA was extracted from bacterial isolates and biopsy specimens (Astrahan' region), using a Probe NA Kit (DNA-Technology, Russia), according to the manufacturer's instructions. DNA was eluted in 50 μL of elution buffer and 5 μL of each DNA solution was used in the PCR.

2.2. VNTR Primer Design

DNA sequences of three *H. pylori*; strains (26695, J99 and G27) deposited in GenBank under accession numbers, NC000915, NC000921 and NC011333 were used to detect the VNTR loci. Analysis using the Tandem Repeat Finder (TRF) program http://tandem.bu.edu was used to identify potential VNTR loci. Primer Premier 5.0 (Premier Biosoft) was used to design PCR primers for amplifying the loci. Primers were designed within the flanking regions, with a theoretical melting temperature of 57°C to 60°C.

2.3. VNTR PCR Amplification

PCR reaction mixture (30 μL) containing 5 μL; of DNA template, 10 pmol of each primer, 1 unit of Taq DNA polymerase, 200 μM of dNTPs and 10 × PCR buffer (500 mM KCl, 100 mM TrisHCl (pH 8.3) 25 mM $MgCl_2$) was utilized. Amplification was carried out in a DNA thermocycler Tercyc (DNA-Technology, Russia) with denaturation at 94°C for 5min, followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 58°C for 45 s and elongation at 72°C for 1 min. A 5-min elongation at 72°C was performed after the last cycle to ensure complete extension of the amplicons. Each PCR product (5 μL) was resolved by 5-8% polyacrylamide; gel electrophoresis with 1X TBE (90 mM Tris-borate, 1 mM EDTA, pH 8). Allelic sizes were estimated using a pBlueScript DNA / MspI (MBI Fermentas, Vilnius, Lithuania) as a size marker. Gels were visualised using UV transillumination and the images captured using the ChemiDoc; System (BioRad).

2.4. Data Analysis

Using the Quantity One 1D Analysis software package (BioRad), the polyacrylamide gel images were analysed and allelic sizes estimated. Allelic sizes were then converted into repeat copy numbers using the formula: Number of Repeats = [Fragment size (bp) – Flanking regions (bp)] / Repeat size (bp). The repeat copy numbers were then rounded down to form whole numbers. When repeat numbers were less than one, they were rounded down to zero, whilst no amplification was represented by the number 99.

3. Results and Discussion

Earlier MLVA method based on 12 VNTR loci was proposed to investigate *H. pylori* from different districts and ethnic groups of China [16]. Tandem repeats were used in range from 12 to 138 bp. Resulting amplicons were detected in agarose gels. We suggested MLVA scheme based only on 4 new VNTR loci with tandem repeats in range from 7 to 12 bp (Table 1).

Table 1. *Characteristic of 4 VNTR loci.*

Loci	Repeat	Repeat size (bp)	No. of allele	Diversity (D)
Hp A	TTTTGATGA	9	6	0.76
Hp D	AAATACAT	8	15	0.92
Hp E	TAATCAC	7	9	0.88
Hp F	AATTCTGTGTTT	12	6	0.57

Suggested scheme was used to differentiate 48 *H. pylori* isolates which originated from various regions (Astrahan' and Rostov region) of Russia. Main characteristic of these loci was described earlier [17]. On the basis of the 4 VNTR loci, the profiles of each isolate were obtained (Figure 1).

The clinical *H. pylori* strains were divided into 48 MTs (MLVA type). Clustering analysis revealed four clusters, with three closely related (A, B, C) and distant cluster D. The greatest part of regional strains belongs to clusters A, B, and C (46/48) with fairly even distribution (Table 2).

Table 2. *Cluster distribution of H. pylori isolates.*

Cluster	Number of strains
A	R – 10/23, A – 13/25
B	R – 4/23, A – 5/25, N – 2/4
C	R – 6/23, A – 6/25
D	R – 2/23, N – 2/4

Thus, the proposed MLVA-typing scheme *H. pylori* allows not only effectively differentiate closely related regional strains, but also to identify genetically remote isolates. The presence of distant cluster D can be explained as follows: it is known that genetic heterogeneity of *H. pylori* within the species is much higher than most other microorganisms [1] and 50 times higher than in humans [18], it was therefore proposed to use the H. pylori as a marker human migration [11]. By the multilocus sequencing-typing (MLST) of; 'housekeeping' genes 370 strains of *H. pylori* from 27

geographical, ethnic and/or linguistic groups of the population were analyzed [11]. The results revealed that *H. pylori* strains are grouped in four modern populations in accordance with their geographical affiliation: two African (hpAfrica1 and hpAfrica2), East Asian (hpEastAsia) and European (hpEurope), which inherited a set of alleles of genes from grandparent populations of Africa, East and Central Asia. Almost all H. pylori strains, isolated in East Asia, were related to the population hpEastAsia. An European population included all *H. pylori* strains, isolated in Europe, and also Turkey, Israel, Bangladesh and Sudan. It was shown earlier that 22 strains of *H. pylori* allocated on the territory of Russia, belong to the same European population [19]. To compare our regional strains with international classification, MTs of 4 strains from data base NCBI were determined *in silico* and included into clustering analysis. *H. pylori* 26695 and *H. pylori* P12 (hpEurope) [20]) were marked in, as N49, 50, and *H. pylori* F16 and XZ274 (hpEastAsia) [20], as N51, 52. Strains *H.pylori* 26695 and *H.pylori* P12 are located in cluster B, and are closely related with the greatest part of regional strains. Strains *H. pylori* F16 and XZ274 are disposed in distant cluster D. To the same cluster two strains from Rostov region belongs, suggesting their Asian origin and belonging to a population hpEastAsia. This fact can be explained by great ethnic diversity of the population of the Rostov region. Thus, regional *H. pylori* strains from the South of Russia are highly heterogeneous, have the similar genotype distribution, and mainly belongs to hpEurope population. At the same time, there were detected strains of hpEastAsia population, due to polyethnic population of the South of Russia. The loci used in this study provided high discriminatory power and successfully separated closely related isolates of different strains from Rostov and Astrahan' region of Russia. The limitation of the most MLVA assays described is the use of agarose gel electrophoresis to separate fragments for allelic sizing, due to inherent inaccuracies of this method to size bands of close molecular weights. So, we used polyacrylamide gel electrophoresis to receive; a higher resolution and accurate determination of repeats number. In contrast with other genotyping methods, the relatively low cost and moderate expertise required for MLVA typing would allow the systematic typing of any new isolate directly by clinical laboratories. All markers proposed here are easy to type with no sophisticated equipment and software. The usefulness of the MLVA typing scheme proposed here must be further determined by investigating a larger population of isolates from Russia and other countries. Further improvements need to be made to the method so that MLVA can be applied directly to biological (biopsies, faeces) and environmental samples, thus avoiding culturing of the pathogen. This would allow epidemiological studies in developing countries where it is not always possible to culture *H. pylori*.

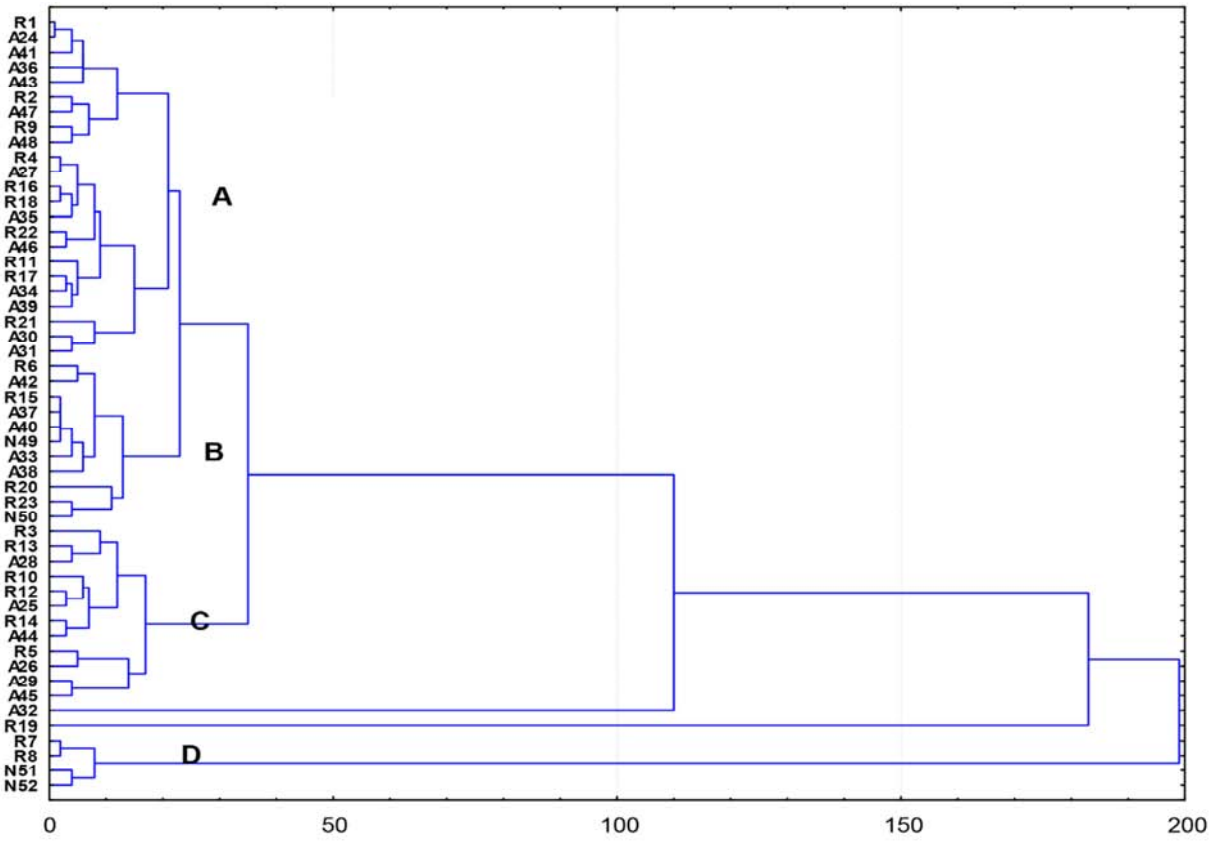

Figure 1. *Dendrogram analysis based on 4 VNTR loci for the 48 H. pylori isolates, including 4 strains from data base NCBI. Clustering analysis of Neighbor-joining tree (N-J) was using the complete linkage. H. pylori isolates from Rostov region were marked in, as R, Astrahan' region, as A, and strains from data base NCBI, as N.*

4. Conclusions

Suggested MLVA typing; of; *H. pylori* may be useful to detect cases of recurrence and reinfection, allows to effectively differentiate closely related regional strains, and also to identify genetically remote isolates in the same population.. Similar study conducted in Russia and Europe for the first time. The proposed; method can be successfully used in molecular epidemiology and geographical classification of *H. pylori*.

References

[1] Achtman, M., T. Azuma, D. E. Berg, Y. Ito, G. Morelli, Z. J. Pan, S. Suerbaum, S. Thompson, A. van der Ende, and L. J. van Doorn. Recombination and clonal groupings within Helicobacter pylori from different geographical regions. Mol. Microbiol, 1999; 32: 459–470.

[2] Takami, S., T. Hayashi, H. Akashi, T. Shimoyama, and T. Tamura. Genetic heterogeneity of Helicobacter pylori by pulse-field gel electrophoresis and re-evaluation of DNA homology. Eur. J. Gastroenterol. Hepatol. 1994. Suppl. 1: S53-S60.

[3] Salaun, L., C. Audibert, G. Le Ley, C. Burucoa, J. L. Fauchere, and B. Picard. Panmictic structure of Helicobacter pylori demonstrated by the comparative study of six genetic markers. FEMS Microbiol. Lett. 1998, 161: 231–239.

[4] Owen, R. J., J. Bickley, M. Costas, and D. R. Morgan. Genomic variation in Helicobacter pylori: application for identification of strains. J. Gastroenterol, 1991, 181: 43–50.

[5] Akopyantz, N., N. O. Bukanov, T. U. Westblom, S. Kresovich, and D. E. Berg. DNA diversity among clinical isolates of Helicobacter pylori detected by PCR-based RAPD fingerprinting. Nucleic Acids Res, 1992, 20:5137–5142.

[6] Gibson, J. R., E. Slater, J. Xerry, D. S. Tompkins, and R. J. Owen. Use of an amplified-fragment length polymorphism technique to fingerprint and differentiate isolates of Helicobacter pylori. J. Clin. Microbiol, 1998. 36:2580–2585.

[7] Ahmed, N., A. A. Khan, A. Alvi, S. Tiwari, C. S. Jyothirmayee, F. Kauser, M. Ali, and C. M. Habibullah. Genomic analysis of Helicobacter pylori from Andhra Pradesh, South India: molecular evidence for three major genetic clusters. Curr. Sci. 2003, 85: 101–108.

[8] Miehlke, S., R. Thomas, O. Gutierrez, D. Y. Graham, and M. F. Go. DNA fingerprinting of single colonies of Helicobacter pylori from gastric cancer patients suggests infection with a single predominant strain. J. Clin. Microbiol. 1999, 37: 245–247.

[9] Nathalie, E., M. van Doorn, F. Namavar, L. J. van Doorn, Z. Durrani, E. J. Kuipers, and C. M. J. E. Vandenbroucke-Grauls. Analysis of vacA, cagA, and IS605 genotypes and those determined by PCR amplification of DNA between repetitive sequences of Helicobacter pylori strains isolated from patients with nonulcer dyspepsia or mucosa-associated lymphoid tissue lymphoma. J. Clin. Microbiol. 1999, 37: 2348–2349.

[10] Ann-Catrin, E. T., N. Hosseini, A. M. Svennerholm, and I. Bo ¨lin. Different Helicobacter pylori strains colonize the antral and duodenal mucosa of duodenal ulcer patients. Helicobacter, 2000, 5:69–78.

[11] Falush D, Wirth T, Linz B, Pritchard JK, Stephens M, Kidd M, Blaser MJ, Graham DY, Vacher S, Perez-Perez GI, Yamaoka Y, Megraud F, Otto K, Reichard U, Katzowitsch E, Wang X, Achtman M, Suerbaum S, Traces of human migrations in Helicobacter pylori populations. Science, 2003, 299:1582-1585.

[12] van Belkum A, Scherer S, van Alphen L, Verbrugh H. Short-sequence DNA repeats in prokaryotic genomes. Microbiol Mol Biol Rev, 1998, 62: 275-293.

[13] Benson G. Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res, 1999, 27: 573-580.

[14] de Knegt, L. V., Pires, S. M., Löfström, C., Sørensen, G., Pedersen, K., Torpdahl, M., Nielsen, E. M. and Hald, T**.** Application of Molecular Typing Results in Source Attribution Models: The Case of Multiple Locus Variable Number Tandem Repeat Analysis (MLVA) of *Salmonella* Isolates Obtained from Integrated Surveillance in Denmark. Risk Analysis, 2016, 36: 571–588. doi:10. 1111/risa. 12483.

[15] Costa, N. S., Pinto, T. C., Merquior, V. L., Castro, L. F., da Rocha, F. S., Morais, J. M.,... & Teixeira, L. M.. MLVA Typing of Streptococcus pneumoniae Isolates with Emphasis on Serotypes 14, 9N and 9V: Comparison of Previously Described Panels and Proposal of a Novel 7 VNTR Loci-Based Simplified Scheme. *PloS one*, 2016, *11* (7), e0158651.

[16] Guo, C., Liao, Y., Li, Y., Duan, J., Guo, Y., Wu,; & Guo, G. Genotyping analysis of Helicobacter pylori using multiple-locus variable-number tandem-repeats analysis in five regions of China and Japan. BMC microbiology, 2011, 11(1), 197.

[17] Sorokin V. M., Pisanov R.V., Bereznyak E. A., Prozorova L. A. Development of multiple-locus variable number of tandem repeat analysis (MLVA) for Helicobacter pylori and its application to Helicobacter pylori isolates from Rostov region, Russia. Rev Electron Biomed / Electron J Biomed .2012; 3: 32-39.

[18] Li W. H., Sadler L. A. Low nucleotide diversity in man. Genetics. 1991, 129: 513–523.

[19] Momynaliev K. T., Chelysheva V. V., Akopian T. A. Population-based identification Russian isolates of H pylori. Genetika, 2005, 41(10):1-4. In Russian.

[20] Kawai et al. Evolution in an oncogenic bacterial species with extreme genome plasticity: Helicobacter pylori East Asian genomes. BMC Microbiology 2011 11:104. doi:10. 1186/1471-2180-11-104.