2. MODERN METHODS OF MOLECULAR DIAGNOSTICS OF INFECTIOUS DISEASES

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GENOMIC DIVERSITY OF NON-TOXIGENIC *VIBRIO CHOLERAE* EL TOR STRAINS AND METHOD FOR DIFFERENTIATION OF CHOLERA VIBRIOS WITH DIFFERENT EPIDEMIC SIGNIFICANCE, USING PCR

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In the territory of the Russian Federation over the period of 2008–2017 in the course of surface water bodies monitoring, 725 non-toxigenic strains of cholera vibrios with *ctxA*$^-$ *tcpA*$^-$ and *ctxA*$^-$*tcpA*$^+$ genotypes were isolated. The question regarding the origins of non-toxigenic strains and their genomic diversity remains an open one. In this context, objective of the study was to investigate genomic diversity of non-toxigenic *V. cholerae* El Tor strains, evaluate their epidemic significance, using a designed multiplex PCR.

We applied conventional microbiological and molecular-genetic methods, as well as whole-genome sequencing tools.

The analysis included isolated natural and experimentally obtained non-toxigenic strains. For non-toxigenic strains the presence of a complete set of mobile genetic elements (MGE), responsible for pathogenic (CTXϕ, TLCϕ, RS1, VPI-1, VPI-2) and epidemic (VSP-I и VSP-II) potential was characteristic. Non-toxigenic strains turned out to be genetically heterogeneous and were divided into three groups. The first group of *ctxA*$^-$*tcpA*$^-$ strains lacked CTXϕ, TLCϕ, RS1, VPI-1, VSP-I, and VSP-II elements. Pathogenicity island, VPI-2, had the deletions the size of 33–49 kb, depending upon the strain. The second group of *ctxA*$^-$*tcpA*$^+$ strains was devoid of CTXϕ, RS1, VSP-I, VSP-II, but preserved pathogenicity islands, VPI-1 with *tcpA,* and VPI-2. The latter one had the deletions the size of approximately 34 kb. The third group consisted of experimentally obtained non-toxigenic strains that lost CTXϕ prophage in aqueous medium, but retained pandemicity islands, VSP-I and VSP-II. Among the studied non-toxigenic strains isolated in the territory of Russia, this type of strains was not found. According to the international NCBI GenBank database, such non-toxigenic strains were detected in endemic as regards cholera regions. Genome analysis of the mentioned strains showed that they were deprived of CTXϕ prophage only, but contained all other MGEs with genes of virulence and epidemicity. The data gathered suggest that natural non-toxigenic *ctxA*$^-$*tcpA*$^+$ strains may be derivatives of toxigenic ones. Thus, in the territory of the Russian Federation two main groups of non-toxigenic strains with *ctxA*$^-$*tcpA*$^-$ and *ctxA*$^-$*tcpA*$^+$ genotypes circulate. Heterogeneity of non-toxigenic *ctxA*$^-$*tcpA*$^+$ strains by the structure of the genome and epidemiological significance pointed to the necessity of PCR construction for their differentiation. We designed multiplex PCR which simultaneously separates toxigenic from non-toxigenic strains by the presence/absence of *ctxA* and *tcpA* genes, and differentiates the latter ones into potentially epidemically hazardous and epidemically safe ones by the presence/absence of pandemicity islands' genes, VSP-I (*VC0180*) and VSP-II (*VC0514*).

Non-toxigenic *V. cholerae* El Tor strains with *ctxA*$^-$ *tcpA*$^+$ genotype are genetically inhomogeneous group with varying epidemiological significance. The strains of *ctxA*$^-$ *tcpA*$^+$VSP$^+$ can pose a potential epidemic threat and circulate only in endemic territory. *ctxA*$^-$*tcpA*$^+$VSP$^-$ strains circulating in the territory of Russia are epidemically safe due to the loss of considerable genome regions.

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DETECTION AND ANALYSIS OF CRISPR-CAS SYSTEMS IN PLASMIDS OF DIFFERENT *BACILLUS THURINGIENSIS* STRAINS

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Bacillus thuringiensis (Bt) is a gram-positive spore-forming bacteria capable of producing toxic proteins (Cry, Cyt or Vip) some of which are used against insects, nematodes and human-cancer cells. CRISPR loci absent in chromosome sequences of Bt strains available in public databases. In 2017 an acting CRISPR-Cas system was detected in its plasmid. The presence and structure of CRISPR-Cas systems in other plasmids of Bt has not yet been studied. The analysis of these systems is basis for research phage resistance in industrially and medically important strains of Bt.

The aim of the study is to perform a search and comparative analysis of CRISPR-Cas systems in plasmids of different strains of Bt using bioinformatic methods.

Nucleotide sequences and protein profiles of all available in NCBI databases (in June 2018) plasmids of Bt have been analyzed by bioinformatic software tools.

We identified the genomic loci of CRISPR-Cas system in 16 circular plasmids ranging in size from 94695 to 761374 bp. 10 plasmids have genes of insecticidal proteins: Cry1Aa, Cry2Aa, Cry2Ab, Cry2Ac. All detected CRISPR loci belong to the class 1, type I, subtype C and vary in length from 3495 to 12188 bp. CRISPR-Cas systems with complete set of *cas*-genes were found in 2 of 16 plasmids. The genes of adaptation module absent in 14 plasmids, therefore an acquisition of new spacers does not occur. One plasmid does not contain CRISPR arrays and gene of *endonuclease Cas3* which cleave foreign genetic elements. CRISPR arrays of 15 plasmids comprise the repeats (32 bp) separated by 3–17 short spacers (32–35 bp). The presence of CRISPR loci in the plasmids confirms a possible transfer of CRISPR locus from the nucleoid to plasmids. The results of this study provide new information about the degradation of CRISPR-Cas system in some Bt strains.

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INDEL TYPING OF *VIBRIO PARAHAEMOLYTICUS* STRAINS ISOLATED DURING OUTBREAKS IN THE RUSSIAN FEDERATION

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The use of molecular methods for intraspecific typing of bacteria allows to analyze and predict the spread

of clones with increased virulence, resistance to environmental factors and antibiotics. One of the new molecular methods is INDEL-typing which is based on the search for spontaneous inserts/deletions of several nucleotides that differ in length in different clones.

Vibrio parahaemolyticus is a common and important pathogen that causes human gastroenteritis worldwide. We have developed a method for typing *V. parahaemolyticus* strains based on the analysis of six INDEL-locus (S.O. Vodop'yanov et al., 2016). The INDEL analyses of the *V. parahaemolyticus* collection revealed that strains of different INDEL-genotypes circulate in environment. The discriminating power of INDEL-typing for environmental strains was 0.95. However, to date, there is no information about the INDEL-genotypes of clinical strains.

The aim of this work was to study INDEL-markers of *V. parahaemolyticus* strains isolated during two foodborne disease outbreaks in the Russian Federation.

It was investigated 29 clinical strains of *V. parahaemolyticus* isolated in July-October 2012 in the Primorsky region of the Russian Federation. The study was performed on INDEL loci Vp967, Vp08, Vp619, Vp2256, VpA472, Vp506. The result showed that all 29 studied *V. parahaemolyticus* strains had identical INDEL genotype with the formula Vp967 — 112, Vp08 — 89, Vp619 — 114, Vp2256 — 111, VpA472 — 95 and Vp506 — 79 base pairs. Thus, both outbreaks were caused by one clone of the pathogen. At the same time, strains with other INDEL genotypes circulated in the environment.

In our opinion, the INDEL-typing method of *V. parahaemolyticus* strains can be useful in carrying out epidemiological investigation of outbreaks of food gastroenteritis.

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EXPRESSION OF RECOMBINANT NS1 PROTEINS OF WEST NILE, DENGUE AND ZIKA FEVER VIRUSES IN *NICOTIANA TABACUM* FOR FUTURE USE IN DIAGNOSTICS

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In connection with the increasing frequency of infectious diseases outbreaks caused by arboviruses, the monitoring of the epidemiological situation in the Russian Federation requires development of immunological diagnostic kits for differential diagnosis. These kits could be developed using individual recombinant antigen proteins of selected viruses. Standard eukaryotic systems, for example insect cells, have a number of limitations in terms of productivity and costs. In our work, we used plants for the production of flavivirus antigens which are an ideal biofabric system because of their ability to generate large amounts of proteins with low cost and to produce an appropriate post-translational modification of recombinant proteins. Protein targets for expression were NS1 non-structural proteins of flaviviruses which were described in the literature as reliable serological markers.

The sequences of the NS1 proteins of Zika virus (ZIKV), West Nile virus (WNV) virus and the two serotypes of Dengue virus (DNV1 and DNV3), have been optimized for expression of the target proteins in the *Nicotiana tabacum*. The resulting DNA sequences were submitted in the GenBank database under accession numbers: MH134590, MH134591, MH134592, MH134593 for ZIKV, DNV1, DV3 and WNV respectively. Sequences were synthesized *de novo* using oligonucleotides by the enzymatic "Two step PCR" method. Expression cassettes containing 35S CaMV promoter and tNOS terminator for strong constitutive expression of the target were constructed on the base of the pBI121 plasmid. Four binary vector systems for the expression of NS1 proteins in plants were developed. Tobacco leaf discs were transformed using *Agrobacterium tumefaciens* Ti-plasmids of strain AGL0 and further regeneration of tobacco plants was carried out. For each expression structure, 10 independent transgenic lines were obtained and were transferred to rooting media for further transfer to conditions of closed soil, which would enable the collection of the necessary amount of biomass to isolate antigen proteins, for their further use in the creation of diagnostic systems. The target gene insertions in each line were confirmed by PCR.

Thus, the plant expression system of West Nile, Dengue and Zika virus antigens was developed and our future studies would include purification of target antigens and their verification as serological markers in diagnostic systems (ELISA, immunochip).

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WHOLE-GENOME SEQUENCING AS A TOOL FOR COMPREHENSIVE ASSESSMENT OF THE PATHOGENIC POTENTIAL OF ANCIENT ARCTIC MICROBIOMES

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Arctic permafrost is a natural reservoir of ancient prokaryotic mobile genetic elements (MGE) associated with pathogenicity or resistance to antimicrobials. It has been shown that ancient MGE have possibility to integration and effective expression in the genomes of modern bacteria. For example, an ancient *Psychrobacter* sp. pKLH80 plasmid from strain isolated in the Pleistocenic permafrost, contains blaRTG-6 β-lactamase gene, able to be mobilized in the modern epidemic *Acinetobacter baumannii* (Petrova M. et al., 2014). Horizontal genetical transfer of virulence and antibiotic resistance determinants from ancient microorganisms can lead to the appearance of genotypes with high epidemic potential. Thus the process of removal of paleomicroorganisms or their genetic material by degradation of permafrost due the global climatic changes is associated with the risk of emergence of new pathogenes or activation of forgotten infectious diseases. An effective monitoring of the pathogenic potential of the polar microbiota should be implemented.

In our opinion, one of the most promising approaches to the study of the pathogenic characteristics of bacteria found in permafrost is the whole genome sequencing. As a result of our team's studies several bacterial genomes isolated from Pleistocenic mammoth fauna were annotated. In particular, the ancient genomes of *Enterococcus* sp. (GenBank Acc. No. LGAN000000000000, NZ_LGAO00000000), *Arthrobacter* sp. ((Acc. No. QDAE00000000), *Clostridium perfringens* (Bac. No. QDAE00000000, QDAF00000000), *Serratia* spp. (Acc. No. MQRH00000000, MQML00000000), *Acinetobacter lwoffii* (Acc. No. LZDF00000000) were described.

The presence of of the modern epidemic clones markers in the genomes of Arctic paleobacteria was found. For example, IS16 genetic element characteristic for modern vancomycin-resistant enterococci in the ancient *E. faecium*