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