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Analysis of the gene structure of antiphage systems of non-toxigenic strains of *Vibrio cholerae* O1 biovar El Tor

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Abstract

Introduction. The presence and structure of antiphage systems that contribute to the resistance of cholera vibrios to lytic phages in non-toxigenic strains of *Vibrio cholerae* O1 biovar El Tor isolated in the Russian Federation and neighboring countries has not been studied.

The aim of the study is the detection and analysis of antiphage systems of non-toxigenic strains of *V. cholerae* O1 biovar El Tor.

Materials and methods. The study involved 126 non-toxigenic (*ctxAB*$^-$*tcpA*$^+$ and *ctxAB*$^-$*tcpA*$^-$) strains of *V. cholerae* O1 El Tor isolated from 1972 to 2018. DNA sequencing was performed on the MGI DNBSEQ-G50 platform. For bioinformatics analysis, the following programs were used: fastp v. 0.23, unicycler v. 0.4.7, Blast 2.16.0, MEGA X, CRISPRCasty-per and CRISPRCasFinder.

Results. Phage-inducible islands of the PLE, BREX and DISARM systems were not detected in the genome of the studied strains. It was found that 80% of *ctxAB*$^-$*tcpA*$^+$ strains contain the type I restriction-modification system, while this system was not detected in *ctxAB*$^-$*tcpA*$^-$ isolates. The genes of the CBASS system were detected in single strains of both groups. In the genome of 35 (32%) studied *ctxAB*$^-$*tcpA*$^-$ strains isolated in different regions of the Russian Federation and neighboring countries, the presence of the CRISPR–Cas system of class 1 types I (subtypes I-E, I-F, I-C) and III (subtype III-B) was established. The number of spacers in this system varied from 0 to 80 and their sequence was homologous to the protospacer regions of DNA of lytic and temperate phages, transposons, plasmids of *V. cholerae*, representatives of the genus *Vibrio* and unrelated bacteria. The presence in a number of strains of spacers homologous to the genetic material of the phage circulating in endemic territories may indicate the imported nature of these strains.

Conclusion. The heterogeneity of the studied non-toxigenic strains of *V. cholerae* O1 El Tor in the presence of antiphage systems was revealed, which expands the information on their genetic organization. In their genome, restriction-modification systems of type I (*ctxAB*$^-$*tcpA*$^+$), CBASS (*ctxAB*$^-$*tcpA*$^+$ and *ctxAB*$^-$*tcpA*$^-$) and CRISPR–Cas class 1 types I (subtypes I-E, I-F, I-C) and III (*ctxAB*$^-$*tcpA*$^-$) were identified. The detection of several types and subtypes of the CRISPR–Cas system in the genome of a number of *ctxAB*$^-$*tcpA* strains may indicate its repeated acquisition through horizontal transfer.

Keywords: *non-toxigenic strains of Vibrio cholerae, antiphage systems, CRISPR–Cas system class 1*

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Оригинальное исследование
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Анализ генов антифаговых систем нетоксигенных штаммов *Vibrio cholerae* O1 биовара Эль Тор

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Аннотация

Актуальность. Наличие и структура антифаговых систем, способствующих устойчивости холерных вибрионов к литическим фагам, у нетоксигенных штаммов *Vibrio cholerae* O1 биовара El Tor, выделенных на территории России и сопредельных стран, не изучена.

Цель — выявление и анализ антифаговых систем нетоксигенных штаммов *V. cholerae* O1 биовара El Tor.

Материалы и методы. В работе использовали 126 нетоксигенных (*ctxAB*$^{-}$*tcpA*$^{+}$ и *ctxAB*$^{-}$*tcpA*$^{-}$) штаммов *V. cholerae* O1 El Tor, изолированных с 1972 по 2018 г. Секвенирование ДНК проводили на платформе MGI DNBSEQ-G50. Для биоинформатического анализа применяли программы fastp v. 0.23, unicycler v. 0.4.7, Blast 2.16.0, MEGA X, CRISPRCastyper и CRISPRCasFinder.

Результаты. В геноме изученных штаммов не выявлены фагоиндуцируемые острова PLE, BREX и DISARM-системы. Установлено, что 80% *ctxAB*$^{-}$*tcpA*$^{+}$-штаммов содержат систему рестрикции–модификации I типа, у *ctxAB*$^{-}$*tcpA*$^{-}$-изолятов данная система не обнаружена. Гены CBASS-системы выявлены у единичных штаммов обеих групп. В геноме 35 (32%) изученных *ctxAB*$^{-}$*tcpA*$^{-}$-штаммов, выделенных в разных регионах РФ и сопредельных странах, установлено наличие CRISPR–Cas-системы 1-го класса типов I (подтипы I-E, I-F, I-C) и III (подтип III-B). Количество спейсеров в данной системе варьировало от 0 до 80, их последовательность была гомологична протоспейсерным участкам ДНК литических и умеренных фагов, транспозонов, плазмид *V. cholerae*, представителей рода *Vibrio* и неродственных бактерий. Наличие у ряда штаммов спейсеров, гомологичных генетическому материалу фага, циркулирующему на эндемичных территориях, может указывать на завозной характер данных штаммов.

Заключение. Выявлена гетерогенность изученных нетоксигенных штаммов *V. cholerae* O1 El Tor по наличию антифаговых систем, что расширяет сведения об их генетической организации. В их геноме выявлены системы рестрикции–модификации I типа (*ctxAB*$^{-}$*tcpA*$^{+}$), CBASS (*ctxAB*$^{-}$*tcpA*$^{+}$ и *ctxAB*$^{-}$*tcpA*$^{-}$) и CRISPR–Cas 1-го класса типов I (подтипы I-E, I-F, I-C) и III (*ctxAB*$^{-}$*tcpA*$^{-}$). Выявление нескольких типов и подтипов CRISPR–Cas-системы в геноме ряда *ctxAB*$^{-}$*tcpA*$^{-}$-штаммов может указывать на её неоднократное приобретение посредством горизонтального переноса.

Ключевые слова: *нетоксигенные штаммы Vibrio cholerae, антифаговые системы, CRISPR–Cas-система 1-го класса*

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

Every year, during monitoring studies of Russian water bodies for cholera, non-toxigenic strains of *Vibrio cholerae* O1 serogroup El Tor biovar are isolated, lacking the *ctxAB* genes (*ctxAB*$^-$) that encode the biosynthesis of cholera toxin (the main virulence factor of the cholera pathogen). Sometimes strains containing the *tcpA* gene *(ctxAB*$^-$*tcpA*$^+$), which is responsible for the production of the main subunit of toxin-coregulated pili of adhesion (colonization factor), are isolated. Both *ctxAB*$^-$*tcpA*$^-$ and *ctxAB*$^-$*tcpA*$^+$ strains do not cause cholera, but they could be the cause of acute intestinal infections. It has been established that non-toxigenic strains of *V. cholerae O1* El Tor can persist in environmental objects in Russia for a long time, forming clonal complexes, and are also imported from cholera-endemic countries [1–7]. To identify the mechanisms of long-term survival of non-toxigenic strains in the external environment, as well as their ability to cause acute intestinal infections, the molecular genetic characteristics of these strains are being actively studied, various kinds of typing are being performed, and phylogenetic analysis is being conducted. As a result, the presence of genes encoding additional virulence and persistence factors has been identified in the genomes of non-toxigenic strains, which can also act as toxic substances. These include loci that provide motility, are responsible for the biosynthesis of additional toxins, a thermolabile hemolysin, proteases, neuraminidase, type 6 and type 3 secretion system proteins, mannose-sensitive adhesion pili, as well as regulatory proteins that control the transcription of virulence and persistence genes [3, 5, 8]. However, the reasons for the long-term survival of non-toxigenic *V. cholerae* O1 El Tor strains in the external environment are not fully understood.

It should be noted that when in the water of open bodies of water, non-toxigenic *V. cholerae* O1 El Tor vibrios can be attacked by bacteriophages (phages), which are also present in this environment. It has been shown that cholera phages play an important role in the genetic diversity of *V. cholerae* strains [9]. The co-existence of *V. cholerae* and phages and the need for both to survive drive the evolution of both *V. cholerae* and phages [9, 10]. Bacteria acquire various mechanisms of resistance to phages through horizontal gene transfer, while phages, in turn, very quickly develop resistance to many bacterial defense systems. It has been found that genes encoding resistance to phages can make up more than 10% of a bacterial genome [11].

A significant number of antiphage systems located on mobile genetic elements have been identified in toxigenic *V. cholerae* O1 El Tor strains. This includes a gene cluster encoding a type I restriction-modification system (*vc1764–vc1769*) located on the VPI-2 pathogenicity island, whose action is based on the activity of two enzymes: a restriction endonuclease (*vc1765*) and a methyltransferase (*vc1769*) [12]. The VSP-I pathogenicity island contains the CBASS (cyclic-oligonucleotide-based antiphage signaling system) antiphage signaling system, which includes an operon of 4 genes: *dncV, capV, cap2, cap3* [13, 14]. The ICE SXT element contains BREX (Bacteriophage exclusion) and DISARM (Defence Islands System Associated with Restriction-Modification) systems [10, 15, 16].

A significant role in protection against the most common phage in the endemic area, ICP1, is played by Phage inducible chromosomal island-like elements (PLE), of which there are currently 10 types [17–19]. However, toxigenic El Tor vibrios lack the adaptive defense system CRISPR–Cas, which was identified in the genome of non-toxigenic *V. cholerae* O1 El Tor strains circulating in endemic areas [20]. This system includes clustered regularly interspaced short palindromic repeats (CRISPR), spacers (sequences of foreign origin), and *cas* genes encoding proteins with various functions [13]. Currently, the CRISPR–Cas system is classified into 2 classes, 6 types and 33 subtypes based on its mechanism of action, the structure of the CRISPR spacers, and the presence of *cas* genes.

In a Class 1 system (types I, III, IV), a multi-component complex consisting of several Cas proteins bound to crRNA interacts with the target. Class 2 systems (types II, V, and VI) contain only one protein (Cas9, Cas12, or Cas13) that performs all the functions of a multi-protein effector complex.

The classification into types is based on the structure of the effector complexes, with systems of the same type typically including a specific protein unique to that type of system. The types, in turn, are divided into subtypes, which differ in the structure of the CRISPR locus and, in certain cases, in the presence of Cas proteins. It is a fact that many Type I systems contain the *cas3* gene, which encodes the Cas3 helicase-nuclease. Through its helicase activity, it unwinds the foreign DNA double helix, and with the involvement of its nuclease domain, it fragments the foreign genetic material. In type III systems, the Cas10 protein exhibits nuclease activity. All types include the Cas1 and Cas2 proteins, which form a complex and are responsible for the adaptation stage, i.e., the insertion of a new spacer into the CRISPR array [20, 21].

Despite active research on *V. cholerae* antiphage systems, their presence in the genomes of non-toxigenic *V. cholerae* O1 El Tor strains isolated in Russia and neighboring countries has not been studied. Considering the above, the aim of the study was to identify and analyze the antiphage systems of non-toxigenic strains of *V. cholerae* O1 El Tor biovar.

Materials and methods

We studied the nucleotide sequences of the complete genomes of 126 non-toxigenic *V. cholerae* O1 El Tor strains isolated in Russia and neighboring countries from 1972 to 2018. The nucleotide sequences of 30

strains (12 *ctxA⁻tcpA⁺* and 18 *ctxA⁻tcpA⁻*) were obtained from the NCBI GenBank database, and 96 strains (3 *ctxA⁻tcpA⁺* and 93 *ctxA⁻tcpA⁻*) were sequenced in this study. For sequencing, the strains were obtained from the State Collection of Pathogenic Bacteria of the Russian Anti-Plague Institute "Microbe" of Rospotrebnadzor, where they were stored in a lyophilized state.

Genomic DNA was prepared according to the manufacturer's protocol from a sodium merthiolate-treated bacterial suspension using the AxyPrep Bacterial Genomic DNA Miniprep Kit (Axygen Biosciences).

Sequencing was performed on the MGI DNBSEQ-G50 platform (MGI). Libraries were prepared according to a standard protocol using the DNBSEQ-G50RS (FCLPE150) and MGI EasyFast-PCR-FREEFS Library PrepSet (MGI) kits. Quality control of the obtained reads was performed using the fastpv.0.23 program, and contigs were assembled using unicyclerv.0.4.7.

The Blast 2.16.0 algorithm (http://blast.ncbi) and the MEGA X program (or BioEditV. 7.0.9.0) were used for bioinformatics analysis. CRISPR–Cas systems and spacers were identified using the CRISPRCastyper (https://github.com/Russel88/CRISPRCasTyper) and CRISPRCasFinder (https://github.com/dcouvin/CRISPRCasFinder) programs.

Results

When studying the nucleotide sequences of complete genomes of non-toxigenic strains, phage-inducible PLE islands were not found in the genomes of either *ctxAB⁻tcpA⁻* or *ctxAB⁻tcpA⁺* strains. The BREX and DISARM systems were also not detected, as these strains lack the ICE SXT elements.

Analysis of type I restriction-modification system genes (*vc1765*, *vc1769*) among 15 *ctxAB⁻tcpA⁺* strains revealed 12 isolates that matched the toxigenic *V. cholerae* N16961 O1 El Tor reference strain for these genes (**Table**). In *ctxAB⁻tcpA⁻* strains, type I restriction-modification system genes were not detected.

The presence of CBASS system genes (*dncV*, *capV*) was established in 4 strains. In the *ctxAB⁻tcpA⁺* strain of *V. cholerae* 56, the gene data structure did not differ from that of the *V. cholerae* N16961 O1 El Tor reference strain. In *ctxAB⁻tcpA⁻ V. cholerae* strains P-18748, 102, and M-1457, the *capV* gene was intact, and identical non-synonymous point mutations were identified in the *dncV* gene, leading to amino acid sub-

Characteristics of certain non-toxigenic *V. cholerae* O1 El Tor strains and the structure of their anti-phage genes

Strain	Location, year	Source of isolation	Gene structure		Type of CRISPR–Cas systems	Amount of spacers
			vc1765/ vc1769	vc0179 (dncV)/ vc0178 (capV)		
Non-toxigenic *ctxAB⁻tcpA⁺*-strains						
M1395[LQBY01]	Russia, Astrakhan, 1981	Environment	–	–	–	–
56[MWRD01]	Ukraine, Mariupol, 1995	Infected patient	int	int	–	–
866[MWRF01]	Ukraine, Yalta, 1996	Environment	int	–	–	–
85[NEDU01]	Ukraine, Berdyansk, 1999	Infected patient	int	–	–	–
P18778[NIFI01]	Russia, Rostov-on-Don, 2005	Infected patient	–	–	–	–
M1434*, M1436*	Russia, Kalmykia, 2006	Environment	int	–	–	–
M1501[LRAE01]	Russia, Kalmykia, 2011	Infected patient	–	–	–	–
M-1504[VTLN01]	Russia, Kalmykia, 2011, 2012	Environment	int	–	–	–
M-1518[LQZR01]	Russia, Kalmykia, 2011, 2012	Environment	int	–	–	–
M-1524[LQZS01], M1528*	Russia, Kalmykia, 2013	Environment	int	–	–	–
2613[PYCA01], 2687[PYCB01]	Russia, Kalmykia, 2015	Environment	int	–	–	–
124[PYCD01]	Russia, Kalmykia, 2017	Environment	int	–	–	–
Non-toxigenic *ctxAB⁻tcpA⁻*-strain						
M-988[LQBX01]	Turkmenistan, 1972	Environment	–	–	I-F mini	Undetermined
M-658*	Russia, Ufa, 1976	Environment	–	–	I-F mini	1
M-659*	Russia, Salavat, 1976	Environment	–	–	I-E	56
M-1114*	Russia, Saransk, 1977	Infected patient	–	–	I-E I-F mini	13 0
M-1115*	Russia, Saransk, 1977	Infected patient	–	–	I-E I-F mini	13 1
M-1394*	Russia, Kaspiysk, 1979	Environment	–	–	I-E	44

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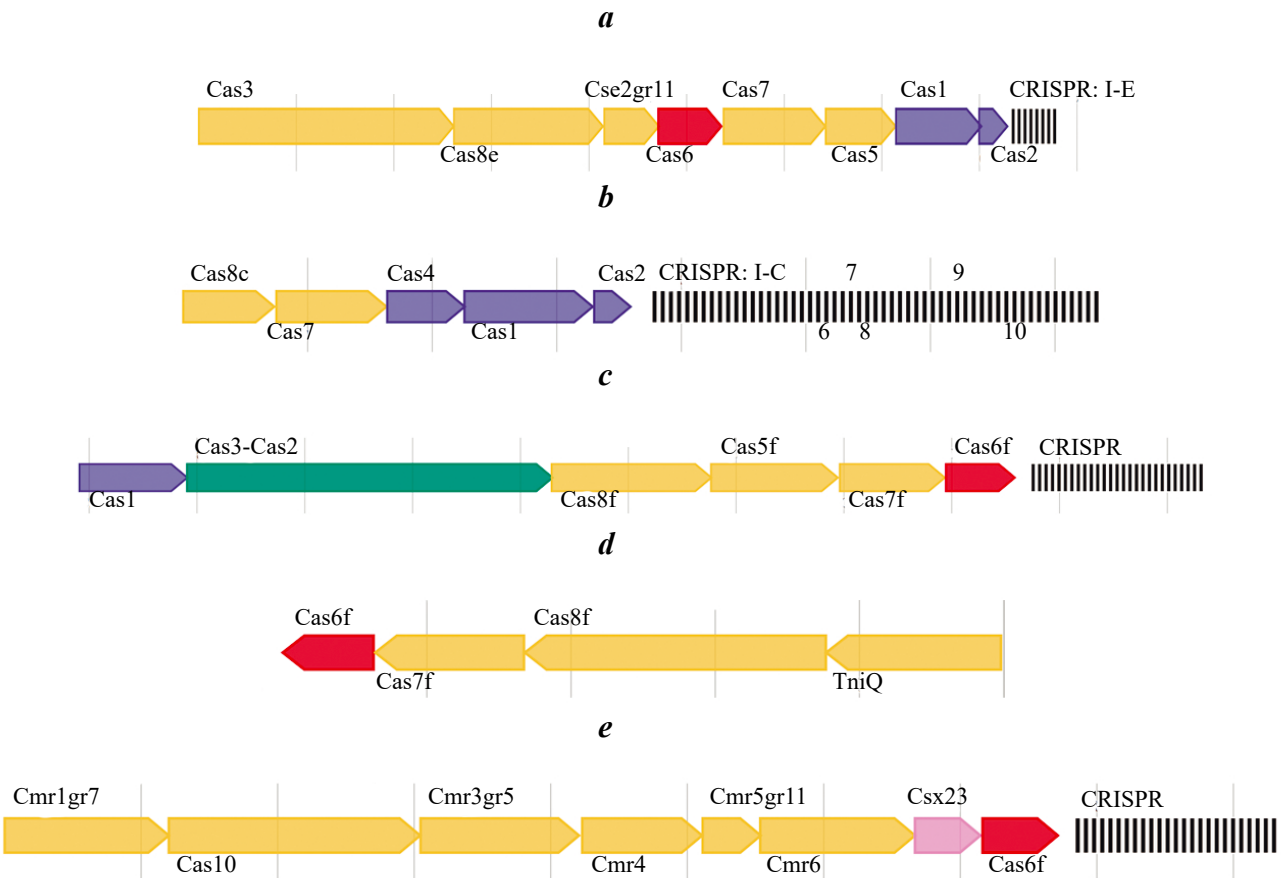
Continuation of the Table

Strain	Location, year	Source of isolation	Gene structure		Type of CRISPR–Cas systems	Amount of spacers
			vc1765/ vc1769	vc0179 (dncV)/ vc0178 (capV)		
M-1222*	Russia, Astrakhan, 1985	Environment	–	–	I-F mini	10
M-1320*	Russia, Saratov, 1998	Environment	–	–	III-B	24
617[NCTY01]	Ukraine, 1999	Infected patient	–	–	I-F** I-E III-B	26 80 25
M1337[NEEB01]	Russia, Astrakhan, 2000	Infected patient	–	–	I-E**	1
M-1388*	Russia, Saratov, 2001	Environment	–	–	I-E I-F mini	13 1
M-1389*	Russia, Saratov, 2001	Environment	–	–	I-E I-F mini	13 1
M-1411*	Russia, Kalmykia, 2002	Infected patient	–	–	I-F mini	1
M-1413*	Russia, Kalmykia, 2002	Environment	–	–	I-C I-F mini	40 2
M-1426*	Russia, Perm Krai, 2003	Environment	–	–	I-F mini	0
M-1428*	Russia, Astrakhan, 2003	Environment	–	–	I-E	44
M-1431*	Russia, Kalmykia, 2005	Environment	–	–	I-E	60
P-18748[NIFH01]	Russia, Sochi, 2004	Infected patient	–	A1003G (S335G) /int	–	–
102[NDXO01]	Ukraine, 2006	Infected patient	–	A1003G (S335G) /int	I-F**	49
M-1441*	Russia, Kalmykia, 2007	Environment	–	–	I-E	48
M-1443*	Russia, Kalmykia, 2007	Environment	–	–	I-E	58
M-1444*	Russia, Kalmykia, 2007	Environment	–	–	I-E	76
M-1447*	Russia, Kalmykia, 2009	Environment	–	–	I-F	15
M-1450*	Russia, Kalmykia, 2009	Environment	–	–	I-F mini	1
M1457[VTLH01]	Russia, Kalmykia, 2009	Environment	–	A1003G (S335G) /int	I-F	2
M-1460*	Russia, Tatarstan, 2010	Environment	–	–	I-E I-F mini	14 1
2403[NEDV01]	Ukraine, 2011	Infected patient	–	–	I-E I-F mini	12 0
M-1486*	Russia, Tatarstan, 2011	Environment	–	–	I-E I-F mini	29 1
M-1487*	Russia, Tatarstan, 2011	Environment	–	–	I-E I-F mini	15 0
M1516[VTZY01]	Russia, Kalmykia, 2012	Environment	–	–	I-F I-C	58 54
M1517[VTZZ01]	Russia, Kalmykia, 2012	Environment	–	–	I-E	23
M-1525*	Russia, Kalmykia, 2012	Environment	–	–	I-F mini	10
M1526[VUAA01]	Russia, Kalmykia, 2012	Environment	–	–	I-F I-C	58 54
29[VUAB01]	Russia, Kalmykia, 2013	Environment	–	–	I-E	7
M-1543*	Russia, Kalmykia, 2017	Environment	–	–	I-E I-F mini	29 1
136[VTLK01]	Russia, Kalmykia, 2018	Environment	–	–	I-E I-F mini	8 3

Note. The strain superscript indicates the NCBI GenBank accession code. *Strains sequenced in this study. "–" — gene(s) not detected; int — the gene structure corresponds to the *V. cholerae* N16961 O1 El Tor reference strain. **The presence of the CRISPR–Cas system was previously established [20].



Structure of CRISPR–Cas systems in the studied *ctxAB*⁻*tcpA*⁻ *V. cholerae* O1 El Tor strains.

a — canonical subtype I-E, present in *V. cholerae* strain 29; *b* — subtype I-C of *V. cholerae* strain M1526; *c* — subtype I-F of *V. cholerae* strain 617; *d* — subtype I-F mini of *V. cholerae* strain M1426; *e* — subtype III-B of *V. cholerae* strain M1320. The spacers (CRISPR) are marked by black vertical lines on the right.

stitutions whose impact on the functional role of the DncV protein is unknown. In the genomes of other strains, the system mentioned was absent (Table).

Next, the presence of the CRISPR–Cas system was investigated. This system was not found in the genomes of *ctxAB*⁻*tcpA*⁺ strains, while among the *ctxAB*⁻*tcpA*⁻ strains, 35 isolates were identified that have a Class 1 Type 2 CRISPR–Cas system — Types I and III. Type I was represented by three subtypes: I-E, I-F, I-C, while Type III was represented by one (III-B). The largest number of strains (20 isolates) were found to have a subtype I-E CRISPR–Cas system, of which 10 isolates had only this subtype, while the others had additional systems. Thus, strain *V. cholerae* 617, in addition to I-E, had 2 more systems (Table). It should be noted that the I-E system structure was canonical in all strains and consisted of 8 *cas* genes (*cas3, cas8e, cse2, cas6, cas7, cas5, cas1, cas2*) (**Figure,** *a*).

Three strains — *V. cholerae* M1413, M1516, M1526 — belonged to subtype I-C (Table, Figure, *b*). Five strains (*V. cholerae* 617, 102, M1457, M1516, M1526) contained a complete subtype I-F CRISPR–Cas system with 6 *cas* genes (*cas1, cas3, cas8f, cas5, cas7, cas6f*: Table, Figure, *c*). A truncated I-F system (*cas6f, cas7f, cas8f*), consisting of 3 *cas* genes, which we have designated I-F mini (Figure, *d*), was identified in a number of strains. It should be noted that this system lacks the *cas3* gene. Two strains, *V. cholerae* M1320 and 617, were type III, subtype III-B (*cmr1, cas10, cmr3, cmr4, cmr5, cmr6, csx23, cas6f*; Table, Figure, *e*)

Non-toxigenic *ctxA*⁻*tcpA*⁻ strains with the CRISPR–Cas system are quite widespread in Russia. These strains were isolated in different years in the Republics of Tatarstan, Dagestan, Bashkortostan, Mordovia, Kalmykia, as well as in the Astrakhan, Saratov regions and the Perm Krai (Table).

The next stage of the study was dedicated to identifying spacers. They were present in almost all systems, with their number varying from 1 to 80, and the largest number of spacers was found in system I-E. The exception was 4 strains containing the I-F mini system, in which spacers were absent (Table). Due to the poor quality of the whole-genome nucleotide sequence presented in GenBank, it was not possible to reliably identify the spacers in strain M-988. The identified spacers were homologous to the protospacer sequences of a large number of lytic and temperate phages, as well as

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plasmids and the *V. cholerae* transposon (phages: O395, VPUSM 8, K139, K491, K571, K575, VcP032, Kappa, Rostov 7, X29, phi 2, JSF1, JSF2, JSF4, JSF5, JSF6, JSF13, JSF14, JSF17, VMJ710, Rostov M3, CP-T1, 24, vB_VchM-138, vB_VchM_VP-3213, Ch457, E8498, fs1, fs2, Vb_VaM_Valp1, ICP1, VRU, VP24-2_Ke, VMJ710, VcP032, VEJphi, VSK, VSKK, ND1-fs1, KSF-1phi, VGJphi, 1.178). O.J. L 286. 45. E12, 1.028. O.J. L 286. 45. B6, 1.159. O.J. L 261. 46. F12, Martha 12B12, Jenny 12G5, vB_Vipa26, vB_Vipa10, vB_Vipa4291, vB_Vipa71, vB_VpS_PG07, Zoerhiza.4_15, 13VV501A, 6E35-1b, D481, D483, D485, D491s, D527, VaK; plasmids: HDW18, pSA7G1, pSA7G2; transposon Tn7005). Furthermore, spacers homologous to the nucleotide sequences of phages and plasmids from *Vibrio* genus (*V. alginolyticus, V. vulnificus, V. parahaemolyticus, V. fluvialis, V. furnissii, V. nigripulchritudo, V. metschnikovii*), as well as from unrelated bacterial species (*Klebsiella* spp., *Escherichia* spp., *Salmonella* spp., *Shigella*, *Shewanella algae*, *Xanthomonas*, *Stenotrophomonas*) were identified. It is worth noting the presence of spacers in certain strains (*V. cholerae* 102, M1428, M1431, M1443, M1444, M1457, M1460, M1486) that are identical to DNA sequences of phage ICP1 (the most common bacteriophage in endemic areas), isolated in different years in the Democratic Republic of Congo and Bangladesh.

Thus, the following antiphage systems were identified in the genomes of the studied non-toxigenic *V. cholerae* O1 El Tor strains: type I restriction-modification (*ctxAB⁻tcpA⁺*), CBASS (*ctxAB⁻tcpA⁺* and *ctxAB⁻tcpA⁻*) and class 1 CRISPR–Cas (*ctxAB⁻tcpA⁻*).

Discussion

Given that non-toxigenic *V. cholerae* O1 El Tor strains circulate in open water bodies, which are also habitats for various cholera phages, it was expected that a large number of antiphage systems would be found in their genomes. However, these strains lack the PLE, BREX, and DISARM antiphage islands of the system. Type I restriction-modification systems, which were found in the genome of *ctxAB⁻tcpA⁺* strains, were not detected in *ctxAB⁻tcpA⁻* strains. CBASS system genes are present in a few strains from both groups. Meanwhile, 35 *ctxAB⁻tcpA⁻* strains studied, isolated from aquatic environments in various regions of Russia and neighboring countries, contain a Class 1 CRISPR–Cas system of types I and III, which is absent in *ctxAB⁻tcpA⁺* isolates. However, subtypes I-C and III-B were detected in only a few strains, while 57% of the strains have the canonical type of the type I-E system. Our data confirm previously obtained information from the analysis of strains circulating in the endemic area regarding the widespread distribution of the I-E system among non-toxigenic *V. cholerae* strains [20]. A number of authors suggest that the stable maintenance of the I-E system structure is due to its location on the GI-24 genomic island, and its transfer to other strains occurs only as part of this mobile genetic element [20, 22]. Certain strains also included a complete I-F system, which is part of the newly discovered VPI-6 (Vibrio Pathogenicity Island), capable, similar to GI-24, of being entirely excised from the chromosome and transferred to other cells [23]. At the same time, most strains had the I-F mini system, which has the *tniQ* gene responsible for transposase production located next to the *cas* gene loci. According to literature data, such systems are associated with the Tn7 transposon [20]. Given the absence of the *cas3* gene encoding a helicase-nuclease in this system, it can be assumed that I-F mini is non-functional.

Since new spacers are primarily inserted into the 5' regions of the system, CRISPR represents a chronological record of the bacterium's interaction with mobile genetic elements. In this regard, the presence of spacers homologous to the genetic material of phage ICP1 circulating in endemic areas may indicate the imported nature of these strains. It is also evident that by protecting *V. cholerae* from predation by cholera phages, as well as phages of other bacteria, the CRISPR–Cas system increases the survival of non-toxigenic strains in the external environment. Perhaps its presence is one of the mechanisms for the long-term circulation of non-toxigenic strains in open water bodies.

Thus, the studied non-toxigenic *ctxAB⁻tcpA⁺* and *ctxAB⁻tcpA⁻ V. cholerae* O1 El Tor strains isolated in Russia and neighboring countries lack a number of mobile genetic elements with anti-phage loci (PLE islands, ICE SXT elements with BREX and DISARM systems). At the same time, a type I restriction-modification system was identified in the genome of *ctxAB⁻tcpA⁺* strains, and a CBASS (*ctxAB⁻tcpA⁺* and *ctxAB⁻tcpA⁻*) and a class 1 CRISPR–Cas system (*ctxAB⁻tcpA⁻*) were identified in one strain.

Conclusion

The research conducted has established the heterogeneity of the studied non-toxigenic *V. cholerae* O1 El Tor strains circulating in Russia and neighboring countries in terms of the presence of antiphage systems located on mobile genetic elements, which expands our knowledge of their genetic organization. It was found that 80% of the studied *ctxAB⁻tcpA⁺* strains contain a type I restriction–modification system, which was not detected in *ctxAB⁻tcpA⁻* strains. Single strains (1 *ctxAB⁻tcpA⁺* and 3 *ctxAB⁻tcpA⁻*) have a CBASS system, which is intact in the *ctxAB⁻tcpA⁺* isolate and corresponds to the toxigenic reference strain *V. cholerae* N16961 O1 El Tor. A class 1 CRISPR–Cas system of type I (subtypes I-E, I-F, I-F mini, I-C) and type III (subtype III-B), which is absent in *ctxAB⁻tcpA⁺* strains, was identified in the genome of 32% of the ctxAB-tcpA- strains studied. The most common (57%) is the canonical system of subtype I-E. The presence of multiple types and

subtypes of the CRISPR–Cas system in the genome of several strains may indicate its repeated acquisition by these isolates through horizontal transfer. Analysis of spacers in the CRISPR cassette allows for the identification of non-toxigenic *V. cholerae* O1 El Tor strains imported from cholera-endemic areas.

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