DOI: https://doi.org/10.17816/EID631523

Development of an INDEL typing system for *ctx+* strains of *Vibrio cholerae* from the seventh pandemic

Sergey O. Vodopyanov, Alexey S. Vodopyanov

Rostov-on-Don Plague Control Researsh Institute, Rostov-on-Don, Russia

ABSTRACT

BACKGROUND: The seventh cholera pandemic is accompanied by the formation of *Vibrio cholerae* clones with new genetic properties, including those with the ability to spread pandemically and cause diseases with a more severe clinical course. The widespread distribution of such genetic variants of *Vibrio cholerae* and the possibility of their introduction into the territory of the Russian Federation necessitate constant comprehensive monitoring using modern molecular genetic technologies.

AIM: To improve INDEL typing of *ctx+* strains of *V. cholerae* of the seventh pandemic by using additional INDEL loci.

MATERIALS AND METHODS: A bioinformatic analysis of 2105 full-genome sequences of toxigenic *ctxAB+tcpA+* strains of *Vibrio cholerae* O1 El Tor from open databases was carried out in order to search for INDEL loci for molecular typing. Based on the convenience criterion for allele size identification, eight INDEL loci were selected. Three loci have been described previously, and five were identified as a result of this work. The designed primers formed amplicons ranging in size from 67 to 390 base pairs, which made it possible to confidently identify them during gel electrophoresis.

RESULTS: The distribution of alleles formed 11 unique INDEL clusters, which we designated A-K. Based on the number of strains within the clusters, three types of clusters were identified: major (A, B and C) made up 89% of the total number of sequences studied, intermediate (D, E, F, G and H) 10.5% of the genomes. Three minor clusters (I, J and K) were represented by single strains. Four clusters united strains isolated in the 20th century (A — in 1941, F — in 1957, G — in 1993, E — in 1999), and seven clusters — in the 21st century in the period from 2003 to 2016. In the period from 2019 to 2023, representatives of INDEL clusters were active: A, B, D and E.

CONCLUSIONS: The study of the timing of circulation suggested that representatives of different clusters have different epidemic potential, which was manifested in the absence of isolation of strains of some clusters in recent years. A comparative study of INDEL typing with SNP typing in the *in silico* analysis of 378 genomes of strains isolated on the African continent indicates that the proposed INDEL typing method is not inferior to SNP typing in terms of resolution.

Keywords: *Vibrio cholerae*; molecular typing; INDEL loci; INDEL genotyping.

To cite this article:
Vodopyanov SO, Vodopyanov AS. Development of an INDEL typing system for *ctx+* strains of *Vibrio cholerae* from the seventh pandemic. *Epidemiology and Infectious Diseases.* 2024;29(4):285–294. DOI: https://doi.org/10.17816/EID631523

Received: 02.05.2024 **Accepted:** 18.06.2024 **Published online:** 19.07.2024

DOI: https://doi.org/10.17816/EID631523

Разработка системы INDEL-типирования *ctx*+ штаммов *Vibrio cholerae* седьмой пандемии

С.О. Водопьянов, А.С. Водопьянов

Ростовский-на-Дону ордена Трудового Красного Знамени научно-исследовательский противочумный институт, Ростов-на-Дону, Россия

АННОТАЦИЯ

Обоснование. Седьмая пандемия холеры сопровождается формированием клонов холерного вибриона с новыми генетическими свойствами, в том числе обладающих способностью к пандемическому распространению и вызывающих заболевания с более тяжёлым клиническим течением. Повсеместное распространение подобных генетических вариантов *Vibrio cholerae* и возможность их завоза на территорию Российской Федерации обусловливают необходимость постоянного комплексного мониторинга с применением современных молекулярно-генетических технологий.

Цель работы — совершенствование INDEL-типирования *ctx*+ штаммов *V. cholerae* седьмой пандемии путём использования дополнительных INDEL-локусов.

Материалы и методы. Проведён биоинформационный анализ 2105 полногеномных сиквенсов токсигенных *ctxAB+tcpA+* штаммов *Vibrio cholerae* O1 El Tor из открытых баз данных с целью поиска INDEL-локусов для молекулярного типирования. На основе критерия удобства идентификации размера аллелей отобрано восемь INDEL-локусов. Три локуса описаны ранее, а пять были идентифицированы в результате проведённой работы. Сконструированные праймеры формировали ампликоны размером от 67 до 390 пар оснований, что позволило их уверенно идентифицировать при проведении электрофореза в геле.

Результаты. Распределение аллелей сформировало 11 уникальных INDEL-кластеров, обозначенных нами A–K. По количеству штаммов в составе кластеров выявлено три типа кластеров: мажорные (A, B и C) составили 89% изученных последовательностей, промежуточные (D, E, F, G и H) — 10,5% геномов. Три минорных кластера (I, J и K) были представлены единичными штаммами. Четыре кластера объединяли штаммы, выделенные в XX веке (A — в 1941 году, F — в 1957 году, G — в 1993 году, E — в 1999 году), а семь кластеров — в XXI веке (с 2003 по 2016 год). В период с 2019 по 2023 год активность проявляли представители INDEL-кластеров: A, B, D и E.

Заключение. Изучение сроков циркуляции позволило предположить, что представители разных кластеров обладают различным эпидемическим потенциалом, что проявилось в отсутствии выделения штаммов некоторых кластеров в последние годы. Сравнительное изучение INDEL-типирования с приёмом SNP-типирования при анализе *in silico* 378 геномов штаммов, изолированных на Африканском континенте, свидетельствует, что предлагаемый способ INDEL-типирования по разрешающей способности не уступает приёму SNP-типирования.

Ключевые слова: *Vibrio cholerae*; молекулярное типирование; INDEL-локусы; INDEL-генотипирование.

Как цитировать:
Водопьянов С.О., Водопьянов А.С. Разработка системы INDEL-типирования *ctx*+ штаммов *Vibrio cholerae* седьмой пандемии // Эпидемиология и инфекционные болезни. 2024. Т. 29, № 4. С. 285–294. DOI: https://doi.org/10.17816/EID631523

Рукопись получена: 02.05.2024 **Рукопись одобрена:** 18.06.2024 **Опубликована online:** 19.07.2024

ЭКО•ВЕКТОР

BACKGROUND

The seventh cholera pandemic involved the formation of clones of *Vibrio cholerae* with new genetic properties, including those with the ability to spread worldwide and cause diseases with a more severe clinical course [1–3]. The widespread distribution of such genetic variants of *V. cholerae* and the possibility of their occurrence in Russia require continuous comprehensive monitoring using modern molecular genetic technologies that provide accelerated diagnostics and timely identification with determination of the epidemic significance of the cholera pathogen for making prompt management decisions [4–6].

Molecular genetic monitoring of *V. cholerae* is based on the analysis of several well-studied genetic markers. The analysis of variable number tandem repeats (VNTR) and INDEL loci have become widespread. In determining VNTR, a complex and labor-intensive method of VNTR typing of toxigenic *V. cholerae* isolated in various regions worldwide has been proposed and is widely used [7–11]. The high stability of VNTR loci contributes to obtaining valid results [12]. Recently, VNTR typing has been successfully used in combination with whole-genome sequencing [10, 13–15].

The INDEL typing method is simpler and more accessible and includes determining the presence of "insertions–deletions" in various genes [16]. The INDEL and VNTR typing methods has its own advantages; however, the most informative results were obtained by combining the two typing methods [17].

The relatively easy-to-use method of INDEL typing of *V. cholerae* is suitable for studying non-toxigenic strains [17], but cannot be used for intraspecific typing of toxigenic strains, because all *ctx*+ cultures with the used set of INDEL loci form only one INDEL genotype [16].

This study aimed to improve INDEL typing of *ctx*+ strains of *V. cholerae* of the seventh pandemic by using additional INDEL loci.

MATERIALS AND METHODS

This study used data from whole-genome sequencing of toxigenic (*ctxAB+tcpA*+) *V. cholerae* O1 El Tor strains obtained on the Illumina MiSeq platform during the implementation of the strategic initiative of socio-economic development of Russia until 2030 "Sanitary shield of the country is the health safety (prevention, detection, response)" (40 strains), 1531 genomes from the NCBI database, and 534 genomes from the European Nucleotide Archive database, presented in the form of reads, which assembly was performed using the Spades program [18]. Information on the date and place of isolation of the strain was obtained from the attached description [19].

For the analysis, we used our own scripts written in the Java and Python programming languages. Geocoding of the places of strain isolation was performed using the Nominatim API service. The online geographic information system was developed using the programming languages HTML, JavaScript, and PHP. The freely distributed Leaflet library written in JavaScript was used as the core, and maps obtained from the Open StreetMap community were used as cartographic data.

RESULTS

Following bioinformatics analysis, over 40 promising INDEL loci were tested for intraspecific typing of toxigenic strains of *V. cholerae*. Each INDEL locus in the chromosome was represented by two alleles; hence, the allele size could be determined by the results of electrophoresis after the polymerase chain reaction (PCR) in the presence of an *in vitro* culture or *in silico* according to the bioinformatics analysis of the nucleotide sequence from databases. Based on the test results, eight INDEL loci were selected for further study. They included the already described loci (i.e., *1095* [20], *3186* [16], and *rtxA4* [21]) and those identified in this study (i.e., 2566, 0667, 1446, 730, and 834). Primers were designed for the identified loci (Table 1), which formed amplicons of 67–390 base pairs in size when conducting PCR, which allowed them to be identified during gel electrophoresis. The distribution of alleles of eight INDEL loci among the 2105 studied *ctx*+ genomes formed 11 unique INDEL clusters, which were designated as A–K (Table 2).

Based on the number of strains in the clusters, three clusters (A, B, and C) were major, and they accounted for 89% of the studied sequences. Clusters D, E, F, G, and H were intermediate and constituted 10.5% of the studied sequences. Three clusters (I, J, and K) were represented by single strains (Table 3). INDEL clusters *ctx*+ *V. cholerae* differed in the time of formation. The strains in the first four clusters were isolated in the twentieth century (A in 1941, F in 1957, G in 1993, and E in 1999), whereas the remaining strains, isolated in the twenty-first century (from 2003 to 2016), were included in seven clusters, among which, B and C were two major ones.

DISCUSSION

In 2019–2023, representatives of four INDEL clusters, namely, A, B, D, and E, have been active, with only isolates from clusters A, B, and D recorded over the past 3 years. For representatives of different clusters, the circulation time (isolation period) of the strains ranged from 4 to 21 years. We believe that clusters F and G can be considered "extinct," because no strains with such genotypes have been isolated over the past 20 years (Table 3).

Representatives of major INDEL clusters A, B, and C have caused several serious outbreaks on various continents, indicating that the strains that form these clusters have a high epidemic potential. Thus, epidemic complications in Haiti and propagations to Africa in 2009–2012 were caused by representatives of cluster B, and the outbreak in Yemen was caused by strains of cluster C (Table 4). In 2023, three toxigenic strains were isolated in Russia: one representative

Table 1. INDEL loci for *ctx+ Vibrio cholerae* typing

No.	Locus	Primers	Position in the genome*	Allele size of INDEL loci
1	1095	ccatcagtctgcctctgacac ttcgacaatcgtcagtagcg	CP028828: 1053058–1052964	87/95
2	rtxA4	tgcaactgggtataacccaatggg tggtgtaccaagacgttcgccaa	CP028827: 1533766–1533377	330/390
3	VC2566	tggttatggattgctgcaagt agtcagtgcgtccagcattt	CP028827: 333608–333522	87/171
4	VCA0667	gacggatatgttgcagtcagc ctcccgagatactccatgtacc	CP028828: 606894–606795	88/100
5	VC1446	gcctatcaagcttgcatgtg tggccaaatacggtattgcg	CP028827: 1540677–1540770	94/100
6	3186	agttggagtgccgtcaaca gcagggtgatagacggtgat	CP028827: 212759–212693	67/74
7	VCA0730	ggggatgaagtaaatgttccga aacaactctacgcaggcttg	CP028828: 677974–677879	90/96
8	VCA0834	tcgcgataaaaggtttagtgatc aaggatccacttcgcgtcc	CP028828: 781715–781810	89/96

* Position is indicated according to the reference genome of *Vibrio cholerae* El Tor N16961 (NCBI accession numbers: CP028827 and CP028828).

Table 2. INDEL genotypes of *ctx+ Vibrio cholerae* and allele sizes of eight INDEL loci

INDEL genotype	Sizes of amplicons, base pairs							
	1095	rtxA4	VC2566	VCA0667	VC1446	3186	VCA0730	VCA0834
A	95	390	87	100	94	67	96	96
B	87	390	87	100	94	67	96	96
C	87	330	87	100	94	67	96	96
D	87	330	87	88	94	67	96	96
E	95	390	171	100	94	74	96	96
F	95	390	171	100	94	67	96	96
G	95	390	87	100	94	67	96	89
H	95	390	87	100	94	67	90	96
I	95	390	171	100	100	74	96	96
J	95	390	87	100	100	67	96	96
K	87	389	87	100	94	67	96	96

of cluster A (Rostov-on-Don) and two representatives of cluster D (Tambov, Moscow).

A study of the circulation periods of *ctx+ V. cholerae*, which are part of INDEL clusters (Tables 3 and 4), showed that representatives of different clusters have different epidemic potential. Thus, strains of cluster F that circulated in Indonesia and Australia until 1997 were isolated in the USA as a result of studying three single introductions. This may indicate the "extinction" of this branch, owing to the low epidemic potential of the *ctx+* strains included in it. The low number of pathogen removals from circulation foci indicates a low epidemic potential of strains in the intermediate and minor INDEL clusters E–K. According to this criterion, strains of INDEL clusters A, B, and D are characterized by an increased epidemic potential and may displace previously formed strains [2, 22].

It is interesting to evaluate *in silico* the efficiency of the proposed INDEL typing scheme for *ctx+ V. cholerae* with a similar system based on the use of SNP analysis of whole-genome sequences.

Analysis of 1757 SNPs revealed three waves and at least eight different phylogenetic lineages of *V. cholerae*, with isolates of the classical biotype forming a separate, highly clustered group, distant from the isolates from El Tor of the seventh pandemic [22, 23]. In the present study, the strains of the major INDEL cluster A may correspond to the strains of wave 1, which

Table 3. Characteristics of the duration of circulation of *ctx*+ strains of *Vibrio cholerae* of various INDEL clusters

No.	INDEL cluster	Number of strains in the INDEL cluster	Years of strain isolation	Time of active circulation, years
1	A	1284	1941–2023	Active since 1941
2	B	415	2004–2022	19
3	C	161	2012–2018	7
4	D	101	2016–2023	Active since 2016
5	E	55	1999–2021	22
6	F	36	1957–1997	41
7	G	27	1993–2000	8
8	H	15	2003–2017	15
9	I	2	2006–2010	5
10	J	2	2015–2017	3
11	K	7	2011–2014	4

Table 4. Characteristics of the geographical distribution of *ctx*+ strains of *Vibrio cholerae* of various INDEL clusters

No.	INDEL cluster	Characteristics of the main focus	Infection introduction from the main focus
1	A	India, 1941	Pandemic spread on all continents. Introduction in Russia (34 pcs): 1970–1973, 1993–1994, 1999, 2001, 2005, 2011, 2014, 2023
2	B	India, 2004	Haiti, 2010–2022; Africa, 2009–2012. The last isolation of 26 strains was in England, 2019. Introduction in Russia: 2010, 2011
3	C	India, 2012	Africa, 2015–2018; Yemen, 2016, 2017
4	D	India, 2016; Bangladesh, 2018 91 isolates of 101 strains were isolated in India and Bangladesh	Iraq, 2017; USA, 2019, 2021–2022; Australia, 2022; Pakistan, 2022; South Africa, 2023 Introduction in Russia: 2023 (Tambov, Moscow)
5	E	Taiwan, 2007 45 out of 55 strains were isolated in Taiwan	South Africa, 2018–2020; China, 2021
6	F	Indonesia, 1957; Australia, 1977–1997 30 of 36 strains were isolated in Australia	USA, 1974, 1978, 1986
7	G	China, 1993	Introduction in Russia: 1999
8	H	Nepal, 2003	Taiwan, 2004–2017; Iraq, 2017; South Korea, 2016
9	I	China, 2006	No introduction
10	J	Congo, 2015	Haiti, 2017
11	K	Nigeria, Togo, 2011	Ghana, 2014

are characterized by the absence of the integrative conjugative element ICE, which distinguishes them from the strains of wave 2 [22]. However, INDEL cluster A includes ICE-positive and ICE-negative isolates. In the future, if necessary, additional INDEL markers to differentiate subgroups within the pandemic INDEL cluster A is recommended.

Subsequently, another study used the same SNP typing technique to analyze 1070 *V. cholerae* O1 strains isolated in 45 African countries over a 49-year period using 9300 single-nucleotide substitutions [24]. The typing scheme was expanded when analyzing 1203 genomes using 9986 SNPs [25]. A subsequent study [26] used 10,679 SNPs, which resulted in the identification of 15 sublineages (AFR1–AFR15). The number of SNPs used was increased to probably increase the method reliability, as cases of significant discrepancies in the results of SNP typing have been described using different sets of SNPs [27].

To compare two different molecular typing methods, a comparative study of cholera outbreaks on the African continent was conducted using the INDEL typing method developed in the current study and SNP typing technique [24, 25] (Table 5).

Table 5. Comparative study of *ctx*+ strains of *Vibrio cholerae* isolated in the African continent by INDEL typing and SNP analysis

Number of strains	Years of strain isolation	INDEL cluster	SNP sublines according to the scheme [24, 25]
322	1981–2017	A	T1-T12
23	2009–2012	B	T12
18	2014–2018	C	T13
7	2018–2020	E	No
1	2015	J	T7
7	2011–2014	K	T12

SNP typing showed that epidemic complications were associated with strains of one extended lineage, which was brought at least 11 times since 1970. Overall, 12 sublineages of *V. cholerae* (T1–T12) were identified using SNP typing; subsequently, sublineage 13 was discovered, which caused a cholera outbreak in Yemen [24, 25].

INDEL typing results showed that the introductions into the countries of the African continent were caused by strains included in eight clusters A, B, C, E, J, and K out of 11 clusters described and not a single SNP lineage. Representatives of INDEL clusters D, F, G, and H in Africa were not detected. The major INDEL cluster A included representatives of all 12 sublineages identified by SNP analysis (T1–T12). The minor INDEL cluster J corresponded to sublineage T7. Sublineage T12 strains were part of INDEL clusters A, B, and K (Table 5). Interestingly, the scheme of Weill in 2017 did not include strains of INDEL cluster E, which caused repeated introductions in South Africa in 2018, 2019, and 2020 (Table 4).

Analysis of strains isolated on the African continent indicated that the proposed method of INDEL typing of *ctx*+ strains of *V. cholerae* is not inferior in resolution to the SNP typing technique [24, 25]. The advantage of the proposed method is the simplicity and clarity of the presentation of the results.

Based on the distribution of eight INDEL loci identified, the geoinformation system "INDEL genotypes of *V. cholerae* O1 strains" was created, located on the geoinformation portal of the Rostov-on-Don Anti-Plague Institute of Rospotrebnadzor (http://gis.antiplague.ru/s_Vcholerae_INDEL.php). The integrated convenient system of filters and search allows for prompt molecular monitoring of *ctx*+ strains from available databases for various requests, displaying the locations of isolation and number of isolated cultures on the map (Fig. 1).

SNP typing is extremely labor-intensive and requires high-quality sequencing and significant computer time to process whole-genome sequencing data. Its result depends on the number of SNPs used. The proposed INDEL typing

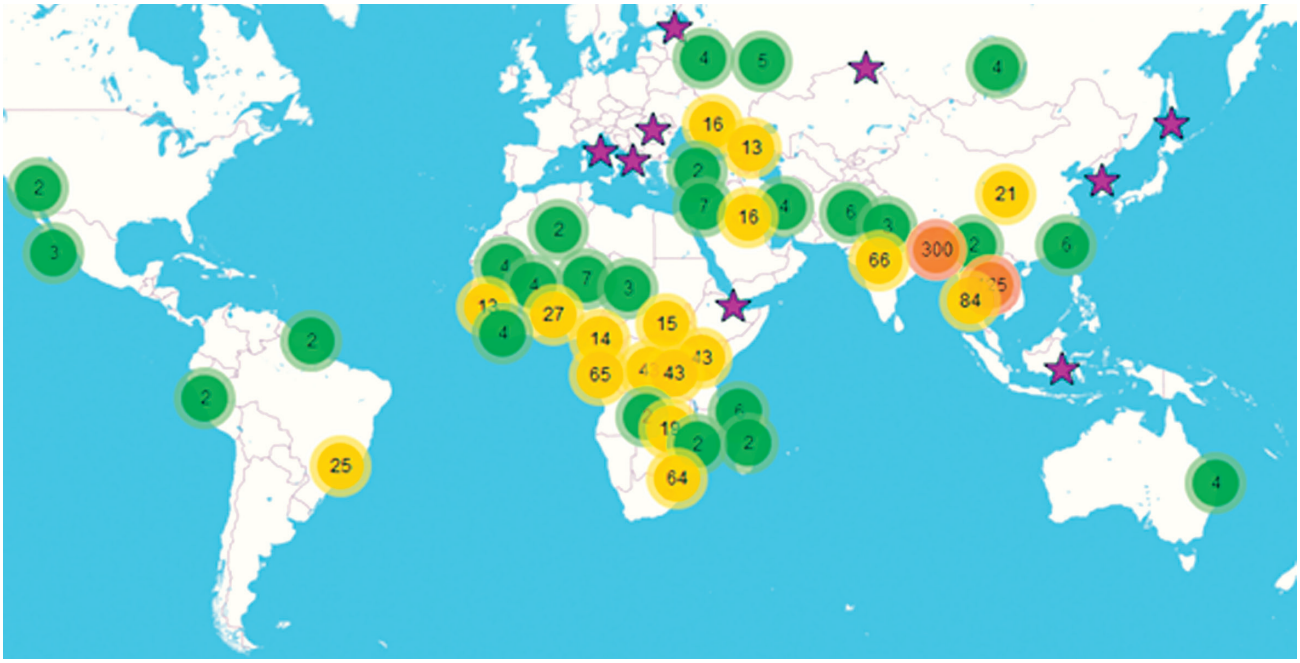

Fig. 1. Appearance of the geographic information system "INDEL genotypes of *V. cholerae* O1 strains". The result of a query on the geographical distribution of strains of the major INDEL cluster A is presented. The circles show the places where the strains were isolated, the number in the circle corresponds to the number of isolated strains. The color of the marker and its shape reflect the number of isolated cultures: single strains — with an star; from 2 to 10 — green circle; from 10 to 100 — yellow circle, over 100 — orange circle.

method at eight loci can be quickly performed using routine PCR, if necessary, without resorting to labor-intensive and expensive sequencing. The advantage of the proposed method of INDEL typing of *ctx+ V. cholerae* is the ability to conduct research *in vitro* in the presence of a pathogen culture and *in silico* when analyzing the whole-genome nucleotide sequence for the purpose of molecular monitoring of cholera.

CONCLUSION

The obtained INDEL typing data enable to consider the *ctx+ V. cholerae* population as a complex biological system characterized by several genetically different INDEL clusters. Moreover, the formation and extinction of individual clusters have been noted [22]. Clusters A and D, which caused the introduction of viruses in Russia in 2023, can be considered active at present. In the future, it is recommended to search for additional loci that potentially allow intracluster typing of major INDEL clusters. Promising targets for analysis may be ICE elements or genes that provide resistance to the toxic effects of heavy metals. Additionally, we believe that studies aimed at identifying new factors and determinants of pathogenicity should focus on representatives of these genetic groups. In this case, representatives of inactive INDEL clusters can be considered as a negative control in a comparative study of genomes.

ADDITIONAL INFORMATION

Funding source. This work was not supported by any external sources of funding.

Competing interests. The authors declare that they have no competing interests.

Authors' contribution. All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work. S.O. Vodopyanov — conducting bioinformation analysis of a collection of strains, writing and editing the text of the article; A.S. Vodopyanov — identification of INDEL markers, writing the text of the article.

REFERENCES

1. Ramamurthy T, Mutreja A, Weill FX, et al. Revisiting the Global Epidemiology of Cholera in Conjuction With the Genomics of Vibrio cholera. *Front Public Health*. 2019;7:203. doi: 10.3389/fpubh.2019.00203 Erratum in: *Front Public Health*. 2019;7:237. doi: 10.3389/fpubh.2019.00237

2. Smirnova NI, Rybalchenko DA, Lozovskiy YV, et al. Analysis of changes in the genome of Vibrio cholerae O1 El Tor genovariants during the current period of the cholera pandemic. *Journal of Microbiology, Epidemiology and Immunobiology*. 2023;100(5):346–357. (In Russ.) doi: 10.36233/0372-9311-389

3. Jubyda FT, Nahar KS, Barman I, et al. Vibrio cholerae O1 associated with recent endemic cholera shows temporal changes in serotype, genotype, and drug-resistance patterns in Bangladesh. *Gut Pathog*. 2023;15(1):17. doi: 10.1186/s13099-023-00537-0

4. Onishchenko GG, Moskvitina EA, Vodop'janov AS, et al. Retrospective Molecular-Epidemiological Analysis of Cholera Epidemic in the Republic of Dagestan in 1994. *Problems of Particularly Dangerous Infections*. 2016;(4):33–41. (In Russ.) doi: 10.21055/0370-1069-2016-4-33-41

5. Noskov AK, Kruglikov VD, Lopatin AA, et al. Results of cholera monitoring in administrative territories of Russia from 2013 to 2019. *Journal of Microbiology, Epidemiology and Immunobiology*. 2021;98(2):163–175. doi: 10.36233/0372-9311-56

6. Mishankin BH, Vodopyanov AS, Lomov YuM, et al. Retrospective VNTR-Analysis of Genotypes of Vibrio Cholerae O1 Strains Isolated, During the 7th Cholera Pandemic, in Rostov Region. *Molecular Genetics, Microbiology and Virology*. 2004;(4):28. (In Russ.) EDN: OJXXPF

7. Danin-Poleg Y, Cohen LA, Gancz H, et al. Vibrio cholerae Strain Typing and Phylogeny Study Based on Simple Sequence Repeats. *J Clin Microbiol*. 2007;45(3):736–746. doi: 10.1128/jcm.01895-06

8. Lam C, Octavia S, Reeves PR, Lan R. Multi-locus variable number tandem repeat analysis of 7th pandemic Vibrio cholerae. *BMC Microbiol*. 2012;12:82. doi: 10.1186/1471-2180-12-82

9. Smirnova NI, Kul'shant' TA, Krasnov YM. MLVA Typing of Clinical Vibrio Cholerae Strains Isolated During Different Periods of the Current Cholera Pandemic. *Molecular Genetics, Microbiology and Virology*. 2015;30(1):15–22. (In Russ.) EDN: TEWRYN

10. Bwire G, Sack DA, Almeida M, et al. Molecular characterization of Vibrio cholerae responsible for cholera epidemics in Uganda by PCR, MLVA and WGS. *PLoS Negl Trop Dis*. 2018;12(6):e0006492. doi: 10.1371/journal.pntd.0006492

11. George CM, Hasan K, Monira S, et al. A prospective cohort study comparing household contact and water Vibrio cholerae isolates in households of cholera patients in rural Bangladesh. *PLoS Negl Trop Dis*. 2018;12(7):e0006641. doi: 10.1371/journal.pntd.0006641

12. Mironova LV, Khunkheeva ZhYu, Basov EA, et al. Analysis of Vibrio cholerae Genotype Stability at Low Temperature and Nutrients Deficiency. *Problems of Particularly Dangerous Infections*. 2016;(3):52–56. (In Russ.) doi: 10.21055/0370-1069-2016-3-52-56

13. Rashid MU, Almeida M, Azman AS, et al. Comparison of inferred relatedness based on multilocus variable-number tandem-repeat analysis and whole genome sequencing of Vibrio cholerae O1. *FEMS Microbiol Lett*. 2016;363(12):fnw116. doi: 10.1093/femsle/fnw116

14. Ambroise J, Irenge LM, Durant JF, et al. Backward compatibility of whole genome sequencing data with MLVA typing using a new MLVAtype shiny application for Vibrio cholerae. *PLoS One*. 2019;14(12):e0225848. doi: 10.1371/journal.pone.0225848

15. Mwaba J, Debes AK, Murt KN, et al. Three transmission events of Vibrio cholerae O1 into Lusaka, Zambia. *BMC Infect Dis*. 2021;21(1):570. doi: 10.1186/s12879-021-06259-5

16. Vodop'yanov AS, Vodop'yanov SO, Oleynikov IP, Mishan'kin BN. INDEL-genotyping of Vibrio cholerae strains. *Epidemiology and Infectious Diseases.* 2017;22(4):195–200. (In Russ.) doi: 10.17816/EID40978

17. Vodopyanov AS, Vodopyanov SO, Oleynikov IP, et al. INDEL- и VNTR-typing Vibrio cholerae strains, isolated in 2013 from the environment objects in the Russian Federation. *Public Health and Life Environment — PH&LE.* 2015;(5(266)):41–44. (In Russ.) EDN: UCHPMN

18. Bankevich A, Nurk S, Antipov D, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol.* 2012;19(5):455–477. doi: 10.1089/cmb.2012.0021

19. Benamrouche N, Belkader C, Njamkepo E, et al. Outbreak of Imported Seventh Pandemic Vibrio cholerae O1 El Tor, Algeria, 2018. *Emerging Infectious Diseases.* 2022;28(6):1241–1245. doi: 10.3201/eid2806.212451

20. Vodop'yanov AS, Vodop'yanov SO, Oleynikov IP, Pisanov RV. Identification of Vibrio cholerae Strains of the «Haitian» Group by PCR Based on INDEL-Typing. *Journal of Microbiology, Epidemiology and Immunobiology.* 2020;97(3):265–270. (In Russ.) doi: 10.36233/0372-9311-2020-97-3-9

21. Monakhova EV, Ghosh A, Mutreja A, Weill F, Ramamurthy T. Endemic Cholera in India and Imported Cholera in Russia: What is Common? *Problems of Particularly Dangerous Infections.* 2020;(3):17–26. doi: 10.21055/0370-1069-2020-3-17-26

22. Mutreja A, Kim DW, Thomson NR, et al. Evidence for several waves of global transmission in the seventh cholera pandemic. *Nature.* 2011;477(7365):462–465. doi: 10.1038/nature10392

23. Monir MM, Islam MT, Mazumder R, et al. Genomic attributes of Vibrio cholerae O1 responsible for 2022 massive cholera outbreak in Bangladesh. *Nat Commun.* 2023;14(1):1154. doi: 10.1038/s41467-023-36687-7

24. Weill FX, Domman D, Njamkepo E, et al. Genomic history of the seventh pandemic of cholera in Africa. *Science.* 2017;358(6364): 785–789. doi: 10.1126/science.aad5901

25. Weill FX, Domman D, Njamkepo E, et al. Genomic insights into the 2016–2017 cholera epidemic in Yemen. *Nature.* 2019;565(7738):230–233. doi: 10.1038/s41586-018-0818-3 Erratum in: *Nature.* 2019;566(7745):E14. doi: 10.1038/s41586-019-0966-0

26. Smith AM, Sekwadi P, Erasmus LK, et al. Imported Cholera Cases, South Africa, 2023. *Emerg Infect Dis.* 2023;29(8):1687–1690. doi: 10.3201/eid2908.230750

27. Kuleshov KV, Vodop'ianov SO, Dedkov VG, et al. Travel-Associated Vibrio cholerae O1 El Tor, Russia. *Emerging Infectious Diseases.* 2016;22(11):2006–2008. doi: 10.3201/eid2211.151727

СПИСОК ЛИТЕРАТУРЫ

1. Ramamurthy T., Mutreja A., Weill F.X., et al. Revisiting the global epidemiology of cholera in conjuction with the genomics of *Vibrio cholera* // Front Public Health. 2019. Vol. 7. P. 203. doi: 10.3389/fpubh.2019.00203 Erratum in: Front Public Health. 2019. Vol. 7. P. 237. doi: 10.3389/fpubh.2019.00237

2. Смирнова Н.И., Рыбальченко Д.А., Лозовский Ю.В., и др. Анализ изменения генома геновариантов *Vibriocholerae* O1 El Tor в современный период пандемии холеры // Журнал микробиологии, эпидемиологии и иммунобиологии. 2023. Т. 100, № 5. С. 346–357. doi: 10.36233/0372-9311-389

3. Jubyda F.T., Nahar K.S., Barman I., et al. Vibrio cholerae O1 associated with recent endemic cholera shows temporal changes in serotype, genotype, and drug-resistance patterns in Bangladesh // Gut Pathog. 2023. Vol. 15, N 1. P. 17. doi: 10.1186/s13099-023-00537-0

4. Онищенко Г.Г., Москвитина Э.А., Водопьянов А.С., и др. Ретроспективный молекулярно-эпидемиологический анализ эпидемии холеры в Республике Дагестан в 1994 г. // Проблемы особо опасных инфекций. 2016. № 4. С. 33–41. doi: 10.21055/0370-1069-2016-4-33-41

5. Носков А.К., Кругликов В.Д., Лопатин А.А., и др. Результаты мониторинга холеры на административных территориях России в период с 2013 по 2019 год // Журнал микробиологии, эпидемиологии и иммунобиологии. 2021. Т. 98, № 2. С. 163–175. doi: 10.36233/0372-9311-56

6. Мишанькин Б.Н., Водопьянов А.С., Ломов Ю.М.,и др. Ретроспективный VNTR-анализ генотипов штаммов *Vibriocholerae* O1, выделенных на территории Ростовской области в годы VII пандемии холеры // Молекулярная генетика, микробиология и вирусология. 2004. № 4. С. 28. EDN: OJXXPF

7. Danin-Poleg Y., Cohen L.A., Gancz H., et al. Vibrio cholerae Strain Typing and Phylogeny Study Based on Simple Sequence Repeats // J Clin Microbiol. 2007. Vol. 45, N 3. P. 736–746. doi: 10.1128/jcm.01895-06

8. Lam C., Octavia S., Reeves P.R., Lan R. Multi-locus variable number tandem repeat analysis of 7th pandemic *Vibrio cholera* // BMC Microbiol. 2012. Vol. 12. P. 82. doi: 10.1186/1471-2180-12-82

9. Смирнова Н.И., Кульшань Т.А., Краснов Я.М. MLVA-типирование клинических штаммов *Vibriocholerae*, изолированных в разные периоды текущей пандемии холеры // Молекулярная генетика, микробиология и вирусология. 2015. Т. 33, № 1. С. 15–22.EDN: TEWRYN

10. Bwire G., Sack D.A., Almeida M., et al. Molecular characterization of Vibrio cholerae responsible for cholera epidemics in Uganda by PCR, MLVA and WGS // PLoS Negl Trop Dis. 2018. Vol. 12, N 6. P. e0006492. doi: 10.1371/journal.pntd.0006492

11. George C.M., Hasan K., Monira S., et al. A prospective cohort study comparing household contact and water Vibrio cholerae isolates in households of cholera patients in rural Bangladesh // PLoS Negl Trop Dis. 2018. Vol. 12, N 7. P. e0006641. doi: 10.1371/journal.pntd.0006641

12. Миронова Л.В., Хунхеева Ж.Ю., Басов Е.А., и др. Анализ стабильности генотипа Vibriocholerae в условиях низкой температуры и дефицита питательных веществ // Проблемы особо опасных инфекций. 2016. № 3. С. 52–56. doi: 10.21055/0370-1069-2016-3-52-56

13. Rashid M.U., Almeida M., Azman A.S., et al. Comparison of inferred relatedness based on multilocus variable-number tandem-repeat analysis and whole genome sequencing of Vibrio cholerae O1 // FEMS Microbiol Lett. 2016. Vol. 363, N 12. P. fnw116. doi: 10.1093/femsle/fnw116

14. Ambroise J., Irenge L.M., Durant J.F., et al. Backward compatibility of whole genome sequencing data with MLVA typing using a new MLVA type shiny application for Vibrio cholera // PLoS One. 2019. Vol. 14, N 12. P. e0225848. doi: 10.1371/journal.pone.0225848
15. Mwaba J., Debes A.K., Murt K.N., et al. Three transmission events of Vibrio cholerae O1 into Lusaka, Zambia // BMC Infect Dis. 2021. Vol. 21, N 1. P. 570. doi: 10.1186/s12879-021-06259-5
16. Водопьянов А.С., Водопьянов С.О., Олейников И.П., Мишанькин Б.Н. INDEL-типирование штаммов *Vibriocholerae* // Эпидемиология и инфекционные болезни. 2017. Т. 22, № 4. С. 195–200. doi: 10.17816/EID40978
17. Водопьянов А.С., Водопьянов С.О., Олейников И.П., и др. INDEL- и VNTR-типирование штаммов *Vibriocholerae*, выделенных в 2013 году из объектов окружающей среды на территории Российской Федерации // Здоровье населения и среда обитания — ЗНиСО. 2015. № 5 (266). С. 41–44. EDN: UCHPMN
18. Bankevich A., Nurk S., Antipov D., et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing // J Comput Biol. 2012. Vol. 19, N 5. P. 455–477. doi: 10.1089/cmb.2012.0021
19. Benamrouche N., Belkader C., Njamkepo E., et al. Outbreak of Imported Seventh Pandemic Vibrio cholerae O1 El Tor, Algeria, 2018 // Emerging Infectious Diseases. 2022. Vol. 28, N 6. P. 1241–1245. doi: 10.3201/eid2806.212451
20. Водопьянов А.С., Водопьянов С.О., Олейников И.П., Писанов Р.В. Выявление штаммов Vibriocholerae «гаитянской» группы с помощью полимеразной цепной реакции на основе INDEL-типирования // Журнал микробиологии, эпидемиологии и иммунобиологии. 2020. Т. 97, № 3. С. 265–270. doi: 10.36233/0372-9311-2020-97-3-9
21. Monakhova E.V., Ghosh A., Mutreja A., Weill F., Ramamurthy T. Endemic Cholera in India and Imported Cholera in Russia: What is Common? // Problems of Particularly Dangerous Infections. 2020. N 3. P. 17–26. doi: 10.21055/0370-1069-2020-3-17-26
22. Mutreja A., Kim D.W., Thomson N.R., et al. Evidence for several waves of global transmission in the seventh cholera pandemic // Nature. 2011. Vol. 477, N 7365. P. 462–465. doi: 10.1038/nature10392
23. Monir M.M., Islam M.T., Mazumder R., et al. Genomic attributes of Vibrio cholerae O1 responsible for 2022 massive cholera outbreak in Bangladesh // Nat Commun. 2023. Vol. 14, N 1. P. 1154. doi: 10.1038/s41467-023-36687-7
24. Weill F.X., Domman D., Njamkepo E., et al. Genomic history of the seventh pandemic of cholera in Africa // Science. 2017. Vol. 358, N 6364. P. 785–789. doi: 10.1126/science.aad5901
25. Weill F.X., Domman D., Njamkepo E., et al. Genomic insights into the 2016–2017 cholera epidemic in Yemen // Nature. 2019. Vol. 565, N 7738. P. 230–233. doi: 10.1038/s41586-018-0818-3 Erratum in: Nature. 2019. Vol. 566, N 7745. P. E14. doi: 10.1038/s41586-019-0966-0
26. Smith A.M., Sekwadi P., Erasmus L.K., et al. Imported Cholera Cases, South Africa, 2023 // Emerg Infect Dis. 2023. Vol. 29, N 8. P. 1687–1690. doi: 10.3201/eid2908.230750
27. Kuleshov K.V., Vodop'ianov S.O., Dedkov V.G., et al. Travel-Associated Vibrio cholerae O1 El Tor, Russia // Emerging Infectious Diseases. 2016. Vol. 22, N 11. P. 2006–2008. doi: 10.3201/eid2211.151727

AUTHORS' INFO

*** Sergey O. Vodopyanov**, MD, Dr. Sci. (Medicine);
address: 117/40 M. Gor'kogo street, 344002 Rostov-on-Don, Russia;
ORCID: 0000-0003-4336-0439;
eLibrary SPIN: 4672-9310;
e-mail: serge100v@gmail.com

Alexey S. Vodopyanov, MD, Cand. Sci. (Medicine);
ORCID: 0000-0002-9056-3231;
eLibrary SPIN: 7319-3037;
e-mail: vodopyanov_as@antiplague.ru

ОБ АВТОРАХ

*** Водопьянов Сергей Олегович**, д-р мед. наук;
адрес: Россия, 344002, Ростов-на-Дону, ул. М. Горького, д. 117/40;
ORCID: 0000-0003-4336-0439;
eLibrary SPIN: 4672-9310;
e-mail: serge100v@gmail.com

Водопьянов Алексей Сергеевич, канд. мед. наук;
ORCID: 0000-0002-9056-3231;
eLibrary SPIN: 7319-3037;
e-mail: vodopyanov_as@antiplague.ru

* Corresponding author / Автор, ответственный за переписку