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E.V. Monakhova[1], A. Ghosh[2], A. Mutreja[3], F.-X. Weill[4], T. Ramamurthy[2]

Endemic Cholera in India and Imported Cholera in Russia: What is Common?

[1]Rostov-on-Don Research Anti-Plague Institute, Rostov-on-Don, Russian Federation; [2]ICMR-National Institute of Cholera and Enteric Diseases, Kolkata, India; [3]Department of Medicine, University of Cambridge, Cambridge, UK; [4]Institut Pasteur, Unité des bactéries pathogènes entériques, Paris, France

Abstract. The endemicity of cholera in India has been well researched. Among the other endemic areas, Indian subcontinent appears to be the cradle of *Vibrio cholerae* genovariants, which subsequently spread worldwide. In contrast, all the cholera cases recorded in Russia are of imported origin. In the past century, such importations might result in epidemics, which, however, ended with elimination of toxigenic *V. cholerae* (TVC) from the affected areas. Currently, the incidence of TVC in water reservoirs or infected returnees from Asian countries are rare events, mostly due to constant surveillance activities. Furthermore, the climatic conditions in the majority of Russian regions are unfavorable for long-term environmental survival of the pathogen. On the other hand, global shifts in climate accompanied by unexpected anomalies in previously stable climatic zones may promote dissemination of imported TVC and emergence of cholera. In some regions of Russia, seasonal weather patterns are pretty similar to Indian. Molecular studies of isolates from diverse territories show that TVC have been introduced into new areas and acquired additional traits, absent in their ancestors.

This article describes genomic characteristics of TVC isolates from Russia, India and some other countries. The review is complemented by bioinformatics analysis of important genetic markers to discriminate between clones that emerged in endemic regions and were imported into cholera-free locations. We have found more evidence in support of primary Indian origin of recent genovariants and their ongoing transformation, including the emergence of a new *rtxA* gene allele with a 60 bp deletion in addition to the previously known null mutation. In certain conditions, TVC could pose a potential threat of spreading epidemic cholera into Russia and other territories. Cholera control requires joint efforts of researchers to learn more about the pathogen at the molecular level for developing effective strategies to protect the humankind throughout the world.

Key words: Vibrio cholerae, genovariants, Haitian strains, endemicity, genetic markers.

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Corresponding author: Elena V. Monakhova, e-mail: monakhova_ev@antiplague.ru.

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Monakhova E.V., ORCID: https://orcid.org/ 0000-0002-9216-7777
Ghosh A., ORCID: https://orcid.org/0000-0003-2323-8735
Mutreja A., ORCID: https://orcid.org/0000-0002-1118-8075
Weill F.-X., ORCID: https://orcid.org/0000-0001-9941-5799
Ramamurthy T., ORCID: https://orcid.org/0000-0001-7999-5036

Е.В. Монахова[1], A. Ghosh[2], A. Mutreja[3], F.-X. Weill[4], T. Ramamurthy[2]

ЭНДЕМИЧНАЯ ХОЛЕРА В ИНДИИ И ЗАВОЗНАЯ ХОЛЕРА В РОССИИ: ЧТО ОБЩЕГО?

[1]ФКУЗ «Ростовский-на-Дону научно-исследовательский противочумный институт», Ростов-на-Дону, Российская Федерация; [2]ICMR-National Institute of Cholera and Enteric Diseases, Kolkata, India; [3]Department of Medicine, University of Cambridge, Cambridge, UK; [4]Institut Pasteur, Unité des bactéries pathogènes entériques, Paris, France

Эндемичность холеры в Индии хорошо изучена. Среди ряда других эндемичных регионов Индийский субконтинент, по-видимому, является местом формирования нескольких вариантов холерных вибрионов, впоследствии распространившихся по всему миру. Напротив, в России все зарегистрированные случаи холеры имели завозное происхождение. В прошлом столетии такие завозы могли приводить к эпидемиям, которые заканчивались вместе с элиминацией токсигенных штаммов *Vibrio cholerae* (TVC) из пораженных регионов. В настоящее время выделение TVC как из водоемов, так и от возвратившихся из азиатских стран больных, является редкостью, в основном благодаря постоянному мониторингу и контролю. Кроме того, климатические условия большинства областей России неблагоприятны для длительного выживания возбудителя во внешней среде. Однако глобальные изменения климата, сопровождающиеся неожиданными аномалиями в ранее стабильных климатических зонах, могут способствовать распространению отдельных завозных TVC, что может привести к эпидосложнениям по холере. В некоторых регионах России сезонные погодные условия очень похожи на индийские. Молекулярные исследования штаммов из разных мест выделения показывают, что TVC распространились на новые территории и приобрели дополнительные свойства, отсутствующие у их недавних предшественников.

В настоящей работе описаны геномные характеристики TVC, выделенных в России, Индии и некоторых других странах. Обзор дополнен биоинформатическим анализом генетических маркеров, значимых для дифференциации клонов, появившихся в эндемичных регионах и завезенных на территории, где холера прежде отсутство-

вала. Мы нашли добавочные свидетельства в пользу первичного индийского происхождения недавно возникших геновариантов и их продолжающейся трансформации, включая появление нового аллеля гена *rtxA* с делецией 60 п.н. в дополнение к ранее известной null-мутации. В определенных условиях TVC могут представлять потенциальную угрозу распространения эпидемической холеры в России и на других территориях. Борьба с холерой требует совместных усилий исследователей, направленных на получение знаний о возбудителе на молекулярном уровне, в целях разработки эффективных стратегий защиты человечества во всем мире.

Ключевые слова: Vibrio cholerae, геноварианты, гаитянские штаммы, эндемичность, генетические маркеры.

Корреспондирующий автор: Монахова Елена Владимировна, e-mail: monakhova_ev@antiplague.ru.

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The problem of cholera remains acute in many developing countries mainly due to poor sanitation and hygiene, inadequate healthcare and lack of surveillance structures. In the developed countries, however, the risk of infection remains extremely low, mainly due to cholera control measures. Nevertheless, mass migration of human population, international travel and maritime movement bring about the danger of importation of toxigenic *V. cholerae* (TVC) to cholera-free locations. Current global shifts in climate accompanied by unexpected anomalies in previously stable climatic zones may promote dissemination of occasionally imported TVC and emergence of cholera outbreaks. The global spread of *V. cholerae* strains with detailed molecular characteristics have been described in numerous publications, but in those studies only a few Russian isolates have been included in the bioinformatics analysis of whole genome sequences (WGSs) [1–5].

While cholera is endemic in the Indian subcontinent, in Russia it is usually associated with the introduction of TVC from abroad. Considering this public health challenge of both the countries, in this article, we focus on the characteristics of TVC isolates from India, Russia and some other affected countries to determine their origin and possible routes of transmission, as well as to estimate the risks of outbreaks in case of new importations. We used relevant published information and performed bioinformatics analysis of certain genes to obtain a comprehensive view on the cholera scenario in Russia and India.

Brief historical account on emergence and spread of cholera: origins and importations

Though severe enteric diseases closely resembling cholera were known in the Indian subcontinent from ancient times, the first recorded cholera epidemic happened in Bengal in 1817. It then spread subsequently to many neighboring Asian countries. All six cholera pandemics of the 19th and 20th centuries, caused by *V. cholerae* of classical biotype, reached Russia entering from the south through the Volga river system causing millions of deaths [6]. Epidemic cholera reached northern and central Europe, including Russia, by 1829. Later, both Americas were affected as well. Although the pathogen was "traveling" from country to country by multiple routes, initial source of infection was primarily undivided India [7].

In spite of the fact that the emergence of the El Tor biotype and the start of the 7th pandemic (7P) has traditionally been attributed to Indonesia [7–10], some "grey areas" remain regarding its primary origin in the absence of sequence information of the "old" strains isolated in different countries. It was presumed that after its initial appearance in the Middle East the ancestor of the 7P lineage migrated to Indonesia, where it acquired the Vibrio seventh-pandemic islands (VSP-I and VSP-II) [9]. Indeed, the two VSPs are absent from the WGSs of Indonesian 1937 isolates (JZCB01, JRBD01 and others), but are present in 1957 Indonesian isolates A6 (CVSG01) and C5 (CP013301), the "oldest" of the 7P strains that could be obtained [4, 11, 12]. However, an "older" strain, A1M (SRR6027668), isolated from a cholera patient in Thailand in 1956, with all El Tor attributes and both VSPs has been recently sequenced and used in genomic analysis [12, 13]. In the light of above finding is seems that the 7P lineage could have emerged in another country and eventually found favorable conditions for epidemic spread from Indonesia in 1961. On the other hand, Pham *et al*. [12] presumed that this strain in fact might be isolated in 1961–1962, as on the phylogenetic tree it grouped with isolates of these years. Unfortunately, till now, there are no more El Tor WGSs of strains (including Indian) isolated before 1961 in NCBI databases, they have not been described in any publications either. Islam *et al*. [14] pointed out that the role of the Middle East and/or Indonesia in the evolution of the 7P strains is highly speculative as it is based on the analysis of a very few genomes. Hence, WGS-based studies using an old collection of strains, if available, is likely to be of much help in identifying the "lost links" in the origin of 7P strains.

During early 1990s, emergence and spread of a new serogroup *V. cholerae* O139 was reported from the Indian subcontinent, which soon disseminated across Asia and reached a number of other countries [10]. However, the expected beginning of the eighth pandemic of O139 cholera did not occur [7].

Tracing the history of cholera in Russia makes it evident that both classical and El Tor as well as O139 strains were imported into Russia from abroad. The last recorded classical cholera epidemic in Russia happened in 1942–1943, caused by an attenuated clone with low epidemic potential due to genomic alterations [15]. The source of importation of this strain was not known, but as during the Second World War people from

many USSR Republics and other territories moved with armies through the country, it is most likely that the mass movement of population would have been the source of the strain. El Tor reached Russia in 1969 and caused a few local outbreaks, but in 1970 and onwards it began to spread intensively into many regions and the Republics of the former USSR. This spread was due to importation of cholera from a number of Asian countries, including India [16]. Since early 2000s, no outbreaks had been recorded in spite of a few sporadic importations through infected returnees from Asia or the rare occurrence of TVC of unknown origin in natural water reservoirs [17, 18]. However, the risk of outbreaks in the future still exists, which demands not only a constant update on cholera morbidity in other countries but also data on the characteristics of dominant pandemic strains.

Endemicity of cholera

Cholera is known to be endemic in the South Asian countries, especially in India and Bangladesh, where infections occur every year [19]. An area is considered to be endemic for cholera, if diarrheal samples from a population there are positive for *V. cholerae* at least in 3 out of the past 5 years [20]. During 1990–2007, 11 of the 28 Indian States were found to be endemic for cholera [21]. A previous report indicated sporadic cholera cases and outbreaks in 35 States in India between 1997 and 2006 [22]. Further analysis of morbidity of cholera during 2010–2015 identified 13 States as endemic [23]. In summary, while the endemic status of certain States in India, such as West Bengal, Maharashtra, Andhra Pradesh appeared to be perennial, in others (Gujarat, Karnataka, Chandigarh, Odisha, Punjab etc.) it could change in different periods of time. Cholera cases appeared sporadically in "non-endemic" States as well. Totally, 78 districts in 15 States were identified as "hotspots" (with an increased risk of the disease) based on observational data and 111 districts in 9 States from model-based predicted number of cases by 2015 [23]. However, from scattered yearly reports, it seems that the entire Indian subcontinent may be considered as endemic for cholera.

In Russia, the previous cholera outbreaks, as well as the more recent sporadic cases, were caused by imported *V. cholerae* O1 El Tor. In the 20th century, such importations occurred frequently, followed by the fast spread of the pathogen, which resulted in outbreaks of cholera with different levels of morbidity in many Eurasian regions of Russia and other USSR Republics; in Ukraine and Siberia in 1970s, in the Far East in 1990s, and in the Republic of Dagestan in 1994 [3, 16, 24]. Only 4 imported *V. cholerae* O139 cholera cases were recorded in 1993 in Rostov region. Of these, 3 were from India, the origin of the remaining one was unknown. However, all isolates had the same genotype as the Indian "prototype O139".

In 2001, an outbreak of El Tor cholera with 52 clinical cases and 18 carriers occurred in Kazan city in the Republic of Tatarstan [3]. Occasional cholera due to infected returnees from Asian countries, presumably from India, was also reported (Bashkortostan, 2004; Tver, 2005; Murmansk, 2006; Moscow, 2010, 2012, 2014). However, cholera has not spread from these sporadic cases. In 2011, an outbreak of cholera took place in neighboring Ukraine (33 patients and 24 carriers) due to visit of tourists and/or seamen. The disease occurred as multiple sporadic cases and did not extend outside Mariupol city; the infection was controlled and eliminated in about 3 months. Nonetheless, more than 50 % of patients displayed severe symptoms of cholera [25, 26].

A number of cases had been imported into the Russian Federation and Ukraine from other Asian countries – Kazakhstan, Tajikistan, China, Bangladesh (via Turkey), Syria and, presumably, from Iran or other Middle East areas [3, 24, 27, 28]. Some of them share endemic status and a few had originated in other endemic regions, like China, which played an important role in the global cholera expansion. Chinese isolates emerged either from endemic sources of TVC within the country or from the transiently imported strains. China, thus, acted as a "sink" or an "amplifier" of epidemic spread [29]. Although unverified, a similar role of non-endemic Asian countries cannot be ruled out. As for the Middle East, it has always played a role in the dissemination of *V. cholerae* due to mass visits of Muslim pilgrims (Hadj) from many countries where cholera is endemic [7, 8]. The 1994 epidemic of cholera in Dagestan happened due to the returnees from Hadj pilgrimage via several Asian countries (Jordan-Syria-Turkey-Iran-Azerbaijan). Retrospective molecular analysis revealed multiple importations of diverse clones at different stages of the epidemic [3].

Toxigenic *V. cholerae* in the environment

An important factor that contributes to cholera endemicity is habitation of *V. cholerae* in the local environments and independence of occurrence of the disease in humans on the importation from outside. The mechanism by which cholera becomes endemic is reliant on the nature of environmental reservoirs [22]. The strategies of survival of *V. cholerae* in natural water sources are governed by associations with phyto- and zooplankton, biofilm formation, transition to non-culturable state etc. [30–32].

In endemic countries, cholera continues to occur due to hot climate, poor sanitation and natural reservoirs of infection. Survival of TVC in such environments is thought to be the possible reason for recurrent annual epidemics in the Indian subcontinent [33]. Several investigations carried out in India during cholera outbreaks showed that TVC were present in samples collected from the affected areas [34–36]. A similar trend was also observed in Russia, where TVC strains were usually found in the environment during the epidemic period and for a short subsequent interval. It has been presumed that Rostov region, Siberia and the Far East have unique ecological niches conducive to the survival of both non-

toxigenic *V. cholerae* and TVC [37]. Considering the epidemics of cholera in the USSR (Astrakhan, Kerch, Rostov-on-Don, Omsk, Barnaul) in the second half of the past century as well as in 2001 (at Kazan), the term "temporary enrooting" was used to describe such intermittent epidemic outbreaks. However, the reason behind the "temporary enrooting" of transient TVC in Russia in the absence of epidemiological evidence still remains elusive. The survival ability of the pathogen outside the human host due to biofilm formation has so far been studied only in experimental conditions, although close to those normally existing in the Russian natural waters [38]. Until now, only one evidence of environmental survival of a toxigenic clone has been known, i.e. isolation of clonal *V. cholerae* strains in Rostov-on-Don from sewage and water from different sites within 2 weeks in 2001. It appears that the pathogen might have originated from patients/carriers and escaped the wastewater treatment process. It is worth mentioning here that even in other cholera endemic regions like Haiti, the concept of environmental TVC and their role in reemerging of cholera after 2010 is not very clear. The sources of outbreaks have not been fully ascertained due to insufficient information. However, it seems reasonable to assume that both water contamination and infected individuals could be the prime factors for the transmission of cholera during outbreaks [39–41].

Based on the genome analysis, epidemic strains circulating in the population during outbreaks are found to be largely distinct from the other *V. cholerae* in the aquatic environment [42, 43]. However, non-toxigenic vibrios seem to help in the emergence of new TVC clones via genetic transfer. Biofilms formed by *V. cholerae* on chitinous surfaces provide favorable niches for recombination events to take place [44]. It was hypothesized, that clonal shifting in *V. cholerae* is driven chiefly by environmental variations rather than by human-borne dissemination [45]. This hypothesis seems relevant at least for endemic countries where TVC are often present in aquatic environments, while its extrapolation to non-endemic areas is yet untimely.

Seasonality, climate, natural disasters and human made calamities

In endemic countries cholera morbidity usually reaches its peak during specific months every year, i.e., displays definite seasonal patterns [14, 46]. For instance, in Bangladesh cholera maintains an annual cycle with two infection peaks – before and just after monsoon, which is linked to changing physico-chemical and biotic factors. These seasonal outbreaks correlate with phytoplankton blooms, which, possibly aid in the increase of cell number of zooplankton-associated TVC [14]. In India epidemic periods also occur during warmer water temperatures in combination with elevated pH and plankton blooms. They are usually linked to pre-monsoon and/or monsoon periods as well. Also, non-seasonal cholera outbreaks occur due to unforeseen sewage leaks from municipality pipelines and contamination of drinking water sources [34, 36, 45]. In addition, cholera outbreaks often follow natural disasters, such as earthquakes, cyclones, tsunami, floods and river erosion [47]. The Haitian epidemic in 2010 also occurred after a massive earthquake [8, 45, 48]. Nevertheless, according to the recent reports (https://www.who.int/wer/en), cholera morbidity has reduced significantly in India.

In Russian Federation and neighboring Ukraine, most recorded epidemic outbreaks of cholera took place during hot seasons and were associated with pollution of natural water reservoirs with sewage or fecal contamination by infected humans [3, 17, 25, 26]. In Stavropol the main cause of cholera outbreak was contamination of a mineral spring by Syrian workers via waste waters [16, 17]. In Tatarstan it happened due to an accident at the gravity collector which resulted in the runoff of sewage into a pond used for bathing (http://healthynation.ru/index.php?view=article&id=821). Although the source of the appearance of TVC in sewage was not defined, it could be a consequence of the importation by Hadj returnees in Tatarstan regions (as in Dagestan in 1994). A similar situation took place in 2014 in Kalahandi district of Odisha, India, where fecal contamination of water reservoir used for recreation led to a large cholera outbreak [49].

Overall there is a vast climatic difference between India and Russia. However, in southern parts of the Russian Federation, the weather conditions closely resemble those of certain Indian States, especially during summer months. Besides, against the backdrop of global warming, climate abnormalities are becoming more frequent with the increase in temperatures even in the northern regions, or the submergence of large flood-free locations due to heavy rainfalls. Seasonal floods, usual for a number of riverine regions, and devastating hurricanes occur every year in different areas in large territory of Russia. Earthquakes, though not regular, have been registered as well. These natural calamities cause severe damage of infrastructures resulting in potable water shortage, unsanitary conditions and thus enhance the possibility of the spread of infectious diseases. Nearly the same consequence may also be caused by technogenic disasters. Therefore, the possibility that the long-term "inter-epidemic" period (from 2002 till today) may come to an end in Russia cannot be totally ruled out, particularly because the currently predominating highly virulent new genetic variants of *V. cholerae* seem to possess better fitness for environmental survival [50].

Molecular insights into the variability of main epidemicity markers in TVC

Regardless of the overall high plasticity of the *V. cholerae* genome, certain mutations in essential genes/gene clusters become conserved in 7P sublineages, which replace each other and come to occupy a dominant position in the epidemiology of cholera. These markers, proposed by a number of authors, include al-

leles of *rstR*, *rstB*, *ctxB* (classical or El Tor type) in the CTX prophages, *tcpA* in the VPI pathogenicity island (prototype El Tor or CIRS101 variants), *rtxA*, encoding the synthesis of the multifunctional-autoprocessing repeat-in-toxin (MARTX) (prototype allele *rtxA1* or truncated *rtxA4*), numbers of heptad repeats (TTTTGAT) at the *ctxAB* operon promoter region (from 3 to 5), mutations in *gyrA* and *parC* genes responsible for quinolone resistance, and structure of the VSP-II island (prototype or with deletion of the central part – VSP-II$^{\Delta 0495\text{-}0512}$) [8, 51–53].

Table 1 summarizes existing information on several genetic traits of toxigenic El Tor strains isolated in the Russian Federation and a few other countries. These data were added to our own analysis results to fill in discrete traits, which are absent in the cited publications. TVC O139 are not included, because our examination of two Russian 1993 isolates when compared with other two strains received from India (1993) and one from Kyrgyzstan (1994) showed the same genotype – *ctxB3*, *rstR*$^{\text{El Tor}}$, *tcpA*$^{\text{El Tor}}$, *rtxA1*, prototype *gyrA*, *parC*, intact VSP-II and 3 heptad repeats. After early 1990s there has been no subsequent importations of O139 serogroup into the Russian Federation.

TVC strains isolated in the last century in Russian Federation from the cholera outbreaks were found to contain prototype genetic traits (including *ctxB3* allele of El Tor type) commonly present in TVC strains worldwide. The last Russian strain of such genotype was isolated in 2000 from river water. Based on the amino acid arrangement in the deduced products of *ctxB* gene of *V. cholerae*, more than ten CT genotypes (*ctxB* alleles) have been reported [10]. *V. cholerae* O1 strains isolated during the Siberia and the Far East cholera epidemics in 1970s, had *ctxB*3 allele, whereas the strains isolated during 1990s possessed *ctxB1* (classical genotype) [24]. Classical *ctxB1* was also identified in the 1990s isolates from the environment, sporadic imported cases, outbreaks in Tatarstan and epidemics in Dagestan. Although this CT genotype was almost displaced all over the world by new sublineages, some of them still managed to continue till 2016, as evidenced by their isolation from 3 indigenous cholera cases associated with raw seafood consumption in South Korea and also from the sea water collected from the coastal area near the affected places (UWOX01, UWOZ01, UWOQ01 UWOY01) [54]. In Bangladesh, during 2013–2014, strains of similar CT genotype (*ctxB1*) briefly outcompeted the currently prevailing lineage (*ctxB7*), which had been dominant in the country since 2008 [55]. The *ctxB1* allele was also shared by strains isolated during 2012–2017 in Iran (ERR2269835-2269838), Iraq (ERR2265655-2265660), Democratic Republic of the Congo (ERR2265650-51) and South Sudan (ERR2265667-2265673) [56].

In early 2000s, the second altered trait accompanying the first one (*ctxB1*) – VSP-II$^{\Delta 0495\text{-}0512}$, was identified in a few Russian environmental isolates. This was followed later by the identification of "CIRS101-like" genotype containing along with *ctxB1* and VSP-II$^{\Delta 0495\text{-}0512}$ new alleles of *tcpA*$^{\text{CIRS101}}$ and *rtxA* with a null mutation G13602A in the strains sporadically imported into the country. Such strains appeared in India in 2004 and within the same year moved to Russia. The SNP in *rtxA* gene in these strains led to the formation of a premature stop codon, completing synthesis of the protein, the C-terminus of which is shortened by 12 amino acid residues (aa). This variant has been designated as *rtxA4* and shown experimentally to have lost its biological activity against eukaryotic cells [57]. It was suggested that the acquired ability to produce CT of classical type makes the functioning of high molecular weight MARTX redundant due to its elevated energy expenditure, and its inactivation is likely to be in favor of energy conservation to ensure rapid cell multiplication and successful dissemination [57].

"Haitian" strains and identification of a new allele of the *rtxA* gene

A new variant of *V. cholerae* El Tor possessing the *ctxB7* allele, which differed from the classical *ctxB1* by an additional mutation at position 58 of *ctxB*, was first identified in the strains that caused the large-scale epidemic in Haiti of 2010 and was designated as Haitian *ctxB* [51]. However, later on it was proved that this new type of *ctxB* allele actually first emerged in Kolkata, India during 2006 and then probably moved to other parts of India and then via Nepal reached Haiti [58]. The 58$^{\text{th}}$ position mutation resulted in His20Asn substitution in the signal peptide of the CtxB7 protein which was shown to play a pivotal role in heightened CT production by "Haitian" strains as compared to other TVCs, hence enhancing their epidemic potential [59]. In 2010, the rare imported cholera cases in Russia as well as the 2011 outbreak in neighboring Ukraine were caused by the "Haitian variant" strains [1, 2, 60, 61]. These strains as well as the other isolates from India, Bangladesh and Russia from 2010 also harbored *tcpA*$^{\text{CIRS101}}$, *rtxA4* and VPSII$^{\Delta 495\text{-}0512}$ (Table 1). Along with these common genetic markers, some traits unique to Haitian isolates, which were absent in the Indian isolates, were described that included 3-nucleotide deletion in the *rstB* gene absent in Indian isolates and a difference in the number of heptad repeats in the *ctxA* promoter regions (4 in Indian and 5 in Haitian strains) [52]. This deletion mutation in *rstB* is a characteristic of the *rstB1* of the RS1 prophage present in most of the El Tor strains. Our analysis of WGSs (data not shown) further confirmed this. Some of the Russian isolates had both *rstR1* (classical type) and *rstR2* (El Tor type), while others, including the Haitian isolates, contained 2 copies of *rstR2*. We further confirmed that they belonged to CTX and RS1 when both prophages were arranged in the same contig (Table 1). Presence of a single copy of prophage in some of the isolates might reflect the incompleteness of their genome assembly. As for the heptad repeats, 5 repeats were found in one isolate imported to Moscow from India in June 2010 [1], before the start of Haitian outbreak (in late October) and also

Table 1

Distribution of genetic markers among *V. cholerae* strains of different origin

Strain	Origin	Gene alleles						VSP-II	TR No.	Ref.
		ctxB	*tcpA*	*rtxA*	*rstR*	*gyrA*	*parC*			
5879[PQBQ01]	Taganrog-72←USSR ←Iran or India	3	ET	1	2x4	pt	pt	pt	4	-
8228[ERR1878614]	Kerch-74←Iran or India	3	ET	1	2	pt	pt	pt	4	4
GP152[CWQK01]	India-79	3	ET	1	2	pt	pt	pt	4	4
14450[ERR1878615], 14451[ERR1878616]	Stavropol'-90←Syria	3	ET	1	2,2	pt	pt	pt	4	4
18252	Rostov-00	3	ET	1	2	pt	pt	pt	4	-
PRL64[CWQM01]	India-92	1	ET	1	2,2	G248T	C254T	pt	4	4
I-1181[LUCN02]	Novosib.reg.-94← Turkey←Bangladesh	1	ET	1	2,1	pt	pt	pt	4	24
I-1187[LYXT02]	Barnaul-94←India	1	ET	1	2	G248T	pt	pt	4	24
I-1263[JPLT02]	Irkutsk reg.-97 ← Kazakhstan	1	ET	1	2	G248T	pt	Δ495-498	3	24,28
R17644[JRTW01]	Achinsk/Krasnoyarsk ter.-97←Kazakhstan	1	ET	1	2	G248T	pt	Δ495-498	3	27
I-1300[JZCC02]	Sakhalin-99←China	1	ET	1	2,1	pt	pt	pt	4	24
16228, 17261,17290	Dagestan-94←Asia	1	ET	1	2,1	pt	pt	pt	4	3
17296	Dagestan-94←Asia	1	ET	1	2	G248T	pt	pt	4	3
18329, 18336	Kazan'-01	1	ET	1	2	G248T	pt	pt	4	3
18337	Kazan'-01	1	ET	1	2,2	G248T	pt	pt	4	3
18847	St.Petersburg-05	1	ET	1	2,1	pt	pt	pt	4	-
18367,18368,18369	Rostov-01	1	ET	1	2	G248T	pt	Δ495-512	4	-
18588	Rostov-03	1	ET	1	2,1	pt	pt	Δ502-512	4	-
CIRS101[ACVW01]	Bangladesh-02	1	CIRS	4	2	G248T	pt	Δ495-512	3	51
M1429[LAEM01]	Beloretsk/Bashkortostan-04←Tajikistan	1	CIRS	4	2	G248T	C254T	Δ495-512	4	27
18826[AYOM01]	Tver-05←Tajikistan	1	CIRS	4	2	G248T	C254T	Δ495-512	4	27
18899[LAKM01]	Murmansk-06←India	1	CIRS	4	2	G248T	C254T	Δ495-512	4	27
0707M[VMQI01]	China-07	1	CIRS	4	2	G248T	C254T	Δ495-512	4	9
301[AJFN02]	Taganrog-11	1	CIRS	4	2	G248T	C254T	Δ495-512	4	18
THSTI_31698[ERR2269927]	India-12	1	CIRS	4	2,2	G248T	C254T	Δ495-512	4	56
81[JRQM01,JPOP01]	Rostov-14	1	CIRS	4	2,2	G248T	C254T	Δ495-512	4	18
IDHO1_726[CWQN01]	India-09	7	CIRS	4	2,2	G248T	C254T	Δ495-512	4	4
TSHSI_02834[ERR1880727] THSTI_02959[ERR1880728]	India-10	7	CIRS	4	2,2	G248T	C254T	Δ495-512	4	4
NHCC-010F[APGD01]	Bangladesh-10	7	CIRS	4	2	G248T	C254T	Δ495-512	4	-
19187[AYNM01]	Moscow-10←India	7	CIRS	4	2	G248T	C254T	Δ495-512	4	60
19188[JNGU01]	Moscow-10←India	7	CIRS	4	2,1	G248T	C254T	Δ495-512	4	60
19191[JNGT01]	Moscow-10←India	7	CIRS	4	2,1	G248T	C254T	Δ495-512	5	60
1792[AELJ01], 1798[AELI01]	Haiti-10	7	CIRS	4	2	G248T	C254T	Δ495-512	5	51,52
2010AA-151[JSTB01]	Haiti-11	7	CIRS	4	2	G248T	C254T	Δ495-512	5	51,52
2012HC-35[JSUD01]	Haiti-12	7	CIRS	4	2	G248T	C254T	Δ495-512	5	-
31[LJFF01], 76[MPVL01], 39[LJFG01], 186[PYBQ01]	Ukraine(Mariupol)-11	7	CIRS	4	2	G248T	C254T	Δ495-512	4	2,61
THSTI_26866[ERR2269923] THSTI_26871[ERR2269924],	India-11	7	CIRS	4	2,2	G248T	C254T	Δ495-512	5	56
THSTI_26907[ERR22699225]	India-11	7	CIRS	4	2,2	G248T	C254T	Δ495-512	4	56
THSTI_32676[ERR2269928]	India-12	7	CIRS	4	2,2	G248T	C254T	Δ495-512	4	56
THSTI_33102[ERR2269929]	India-12	7	CIRS	4a	2,2	G248T	C254T	Δ495-512	4	56
6878[AYNL01]	Moscow-12←India	7	CIRS	4a	2	G248T	C254T	Δ495-512	4	60
W4-13[NIWXO01]	India-13	7	CIRS	4a	2	G248T	C254T	Δ495-512	4	-
THSTI_36124, _36133, _36136, _36268[ERR2269930,44-46]	India-13	7	CIRS	4a	2,2	G248T	C254T	Δ495-512	4	56
CNRVC140176[ERR2265649], THSTI_41049, _41055, _41081[ERR2269947-49]	India-14	7	CIRS	4a	2,2	G248T	C254T	Δ495-512	4	56
3265[JRQL01]	Moscow-14←India	7	CIRS	4a	2	G248T	C254T	Δ495-512	4	61

Red font designates clinical strains; green – environmental, black – unknown origin; WGSs or ERRs from NCBI are supplied by accession numbers as superscripts. ET – El Tor, C – classical, pt – prototype, TR No. – the number of tandem TTTTGAT repeats in the promoter region of *ctxA*. Genes *rstR* are presented as found in WGSs which not necessarily properly reflect their real distribution.

Table 2

Incidence of *ctxB7 tcpA*^{CIRS} *rtx4a* VSP-II$^{\Delta 495\text{-}512}$ 4TTTTGAT genotype in clinical *V. cholerae* strains isolated from 2014 to 2018

Year	*V. cholerae* strains	Country	No.-WHO[□]
2014	CNRVC140176[ERR2265649], THSTI_41049[ERR2269947], _41055[ERR2269948], _41081[ERR2269949]	India	4031/21
2015	IDH08147[SISQ01], THSTI_45544, _45869, _45983, 46073[ERR2269950-53]	India	889/4
	UG026[PYRH01]	Uganda	1461/33
	7714[MSEO01], OO4[MSEN01], 1Mo[MSEM01], 39361[MSIV01] (+18)*	Tanzania	11563/144
	31_1[ERR2265589], 4621STDY6714748, 6714749, 6714750, 6714758[ERS1572783-85,93]	Kenya	13291/67
2016	THSTI_52586, _52588, _52629, _52665[ERR2270655-58]	India	841/3
	CNRVC170168[ERR2265674], 170170[ERR2265676], 170173[ERR2265677], 170174[ERR2265678], 170175[ERR2269613]	Yemen	15751/164
	UG020[PYRG01]	Uganda	516/11
	4621STDY6714780[ERS1572815]	Kenya	5866/80
2017	THSTI_52712, _55199, _56650, _56695[ERR2270659-62]	India	385/3
	Ogawa OG1[WOFA01]	Iraq	NA
	CNRVC170178, 170179[ERR2269616-17], 170185[ERR2269640], 170188, 170188[ERR2269643-44] (+5)*	Yemen	1032481/2276
2018	KOL18B3-1[JACAAD01], KOL18B2-2[JACAAE01]**	India	697/0
	NALMLE-03[SIUE01], -13[SIUO01], -22[SIUE01], -30[SIVF01] (+10)*	Bangladesh	566/0
	2018HL24[WSLE01] (imported)[□□]	China	NA
	YA00120881[JAAOXH01]	Zimbabwe	10692/65

[□]Numbers of cholera cases/deaths reported to WHO (https://www.who.int/wer/en/).
*(Numbers of additional analyzed strains with *rtx4a* from the same outbreaks).
**Strain with 5 TTTTGAT.
[□□]Strain from a cholera case imported from India or Nepal [65].
NA – not available.

in a 2011 Indian isolate. The SNPs in the *gyrA* (G248T) and *parC* (C25T) did not appear to be unique for Haitian strains, but seem to be common in Indian isolates identified in the late 1990s and they became conserved in most of the succeeding isolates [4, 51, 52, 56, this study].

Interestingly, in two clinical Moscow isolates, which were imported from India in 2012 and 2014, a previously undescribed *rtxA* allele was detected with a 60 bp deletion in the proximal part of the gene (without any frame shift) and the corresponding deletion of 20 aa (582-601) in the protein (in addition to the absence of 12C-terminal aa due to the premature stop-codon). This new allele, a variant of the previously described *rtxA4* [57], was designated by us *rtxA4a*. Apparently, the new genovariants are gradually continuing to inactivate MARTX in order to conserve energy. The 60 bp deletion in *rtxA4a* is unlikely to affect gene expression, but possibly could have further suppressed biological activity of the protein and thus reduced excess energy costs to the bacterium. The deletion of 20 aa in RtxA4a falls into the region of N-terminal repeats, whose function for *V. cholerae* MARTX is not precisely established. However, similar deletion in MARTX of *V. vulnificus* completely suppressed not only its biological activity, but also translocation of the toxin into eukaryotic cells [62]. Further studies on the structure of *rtxA* gene in the recent isolates of *V. cholerae* and its relationships with virulence will be of much interest.

The *rtxA* genotype *rtxA4a* was identified in one of the two analyzed Indian 2012 isolates (Table 1). Using Blast search in NCBI databases, we have identified *rtxA4a* allele in more recent isolates of *V. cholerae* (2013–2018) not only from India, but also from a number of Asian and African countries (Table 2). All these isolates belonged to the T13 sublineage of the 7P lineage [56, 63, 64]. This finding suggests that the novel *rtxA4a* allele is an additional attribute of T13 "post-Haitian" outbreak isolates. It is most likely that they were imported from India which has trade and travel connections with many countries listed in Table 2 (https://oec.world). At least, one strain (China, 2018) is known to be isolated from a patient with a recent travel history in Nepal and India [65]. It is still unknown whether such *V. cholerae* strains already reached the Americas, as the Blastn program did not find *rtxA4a* in the available genomes besides those shown in Table 2. However, the Haitian 2011–2012 isolates contained *rtxA4* (Table 1). It should be noted just as, that *ctxB7* allele emerged on the background of *rtxA4*, as the former was never detected in strains with prototype *rtxA1* [57], while our analysis conducted so far has shown that *rtxA4a* most likely appeared on the background of *ctxB7*, because no one strain with *ctxB1* and *rtxA4a* was found. We have analyzed only a few representative isolates from some outbreaks and hence the real proportion of *rtxA4a* in majority of the *V. cholerae* population is not known. Further investigation will reveal its influence in pandemicity or persistence potential of TVC.

Conclusions

Present description of the spread of cholera in India and Russia shows that in spite of different current epidemic situation in two countries, some common traits may be found concerning the risks of the disease upsurge. The emergence of new genotypic variants in

India was rather quickly followed by their migration to Russia, as well as to other countries worldwide, some of which are endemic for cholera. Due to global warming, climatic conditions of certain Russian territories have become warmer, which helps the pathogen in its survival, proliferation and transmission. Russia is also struck by natural disasters which may lead to the shortage of pure water and unsanitary conditions. Though the chances of secondary dissemination of TVC imported to Russia are extremely low owing to the urgent implementation of all the necessary anti-epidemic measures, the influencing factors such as hot weather, heavy rainfalls, plankton blooms, damage of local infrastructure etc., may favor spread of *V. cholerae*. Indisputably, cholera is a global health problem, not only because of its perennial presence in endemic areas, but also its spread in cholera-free countries with well-developed healthcare and anti-epidemic systems. Epidemiological and other control measures are being designed to contribute to the achievement of the goal set by WHO, i.e. total eradication of cholera by 2030 [66]. Molecular studies will promote further progress in certain directions that include checking the persistence of *V. cholerae*, its antimicrobial resistance, development of new diagnostic methods and effective vaccines [10, 67].

Here we used only a restricted set of genetic traits, characteristic of *V. cholerae* El Tor strains isolated in Russia and compared them with those from India and other affected countries while many essential features, such as the presence of additional genomic islands, intact clusters for biofilm formation, sets of housekeeping genes and, especially, the structure of ICE elements, responsible for antibiotic resistance, were not included in our analysis, as this will be the subject of a separate investigation. The markers we selected, while they do allow to distinguish between the representatives of different sublineages, are not adequate in establishing the full picture of the pathogen's evolution. Comparative bioinformatics analysis of WGSs (such as SNP-typing, maximum likelihood phylogeny) is much more discriminative and informative for monitoring of the global spread of the pandemic lineages and hence ought to be carried out with larger number of Russian isolates and compared with Indian and other strains. This review highlights the importance of joint efforts of researchers in India, Russia and in other countries to learn more about the emergent *V. cholerae* strains at the molecular level for the safety of the population in both the countries as well as throughout the world.

References

1. Kuleshov K.V., Vodop'ianov S.O., Dedkov V.G., Markelov M.L., Deviatkin A.A., Kruglikov V.D., Vodop'ianov A.S., Pisanov R.V., Mazrukho A.B., Titova S.V., Maleev V.V., Shipulin G.A. Travel-aassociated *Vibrio cholerae* O1 El Tor, Russia. *Emerg. Infect. Dis*. 2016; 22(11):2006–8. DOI: 10.3201/eid2211.151727.

2. Kuleshov K.V., Kostikova A., Pisarenko S.V., Kovalev D.A., Tikhonov S.N., Savelieva I.V., Saveliev V.N., Vasilieva O.V., Zinich L.S., Pidchenko N.N., Kulichenko A.N., Shipulin G.A. Comparative genomic analysis of two isolates of *Vibrio cholerae* O1 Ogawa El Tor isolated during outbreak in Mariupol in 2011. *Infect. Genet. Evol*. 2016; 44:471–8. DOI: 10.1016/j.meegid.2016.07.039.

3. Onishchenko G.G., Moskvitina E.A., Vodop'janov A.S., Monakhova E.V., Pisanov R.V., Vodop'ianov S.O., Chemisova O.S., Kruglikov V.D., Titova S.V. [Retrospective molecular-epidemiological analysis of cholera epidemic in the Republic of Dagestan in 1994]. *Problemy Osobo Opasnykh Infektsii [Problems of Particularly Dangerous Infections]*. 2016; (4):33–41. DOI: 10.21055/0370-1069-2016-4-33-41.

4. Weill F.-X., Domman D., Njamkepo E., Tarr C., Rauzier J., Fawal N., Keddy K.H. , Salje H., Moore S., Mukhopadhyay A.K., Bercion R., Luquero F.J. , Ngandjio A., Dosso M., Monakhova E., Garin B., Bouchier C., Pazzani C., Mutreja A., Grunow R., Sidikou F., Bonte L., Breurec S., Damian M., Njanpop-Lafourcade B.-M., Sapriel G., Page A.-L., Hamze M., Henkens M., Chowdhury G., Mengel M., Koeck J.-L., Fournier J.-M., Dougan G., Grimont P. A.D., Parkhill J., Holt K.E., Piarroux R., Ramamurthy T., Quilici M.-L., Thomson N.R. Genomic history of the seventh pandemic of cholera in Africa. *Science.* 2017; 358:785–9. DOI: 10.1126/science.aad5901.

5. Greig D.R., Schaefer U., Octavia S., Hunter E., Chattaway M.A., Dallman T.J., Jenkins C. Evaluation of whole-genome sequencing for identification and typing of *Vibrio cholerae*. *J. Clin. Microbiol*. 2018; 56:e00831-18. DOI: 10.1128/JCM.00831-18.

6. Patterson K.D. Cholera diffusion in Russia, 1823–1923. *Soc. Sci. Med.* 1994; 38(9):1171–91. DOI: 10.1016/0277-9536-(94)90183-x.

7. Siddique A.K., Cash R. Cholera outbreaks in the classical biotype era. *Curr. Top. Microbiol. Immunol*. 2014; 379:1–16. DOI: 10.1007/82_2013_361.

8. Mukhopadhyay A.K., Takeda Y., Nair G.B. Cholera outbreaks in the El Tor biotype era and the impact of the new El Tor variants. *Curr. Top. Microbiol. Immunol.* 2014; 379:17–47. DOI: 10.1007/82_2014_363.

9. Hu D., Liu B., Feng L., Ding P., Guo X., Wang M., Cao B., Reeves P.R., Wang L. Origins of the current seventh cholera pandemic. *Proc. Natl. Acad. Sci. USA*. 2016; 113(48):E7730–9. DOI: 10.1073/pnas.1608732113.

10. Ramamurthy T., Mutreja A., Weill F.-X., Das B., Ghosh A., Nair G.B. Revisiting the global epidemiology of cholera in conjunction with the genomics of *Vibrio cholerae*. *Front. Public Health.* 2019; 7:203. DOI: 10.3389/fpubh.2019.00203.

11. Mutreja A., Kim D.W., Thomson N.R., Connor T.R., Lee J.H., Kariuki S., Croucher N.J., Choi S.Y., Harris S.R., Lebens M., Niyogi S.K., Kim E.J., Ramamurthy T., Chun J., Wood J.L., Clemens J.D., Czerkinsky C., Nair G.B., Holmgren J., Parkhill J., Dougan G. Evidence for several waves of global transmission in the seventh cholera pandemic. *Nature.* 2011; 477(7365):462–5. DOI: 10.1038/nature10392.

12. Pham T.D., Nguyen T.H., Iwashita H., Takemura T., Morita K., Yamashiro T. Comparative analyses of CTX prophage region of *Vibrio cholerae* seventh pandemic wave 1 strains isolated in Asia. *Microbiol. Immunol.* 2018; 62(10):635–50. DOI: 10.1111/1348-0421.12648.

13. Nguyen T.H., Pham T.D., Higa N., Iwashita H., Takemura T., Ohnishi M., Morita K., Yamashiro T. Analysis of Vibrio seventh pandemic island II and novel genomic islands in relation to attachment sequences among a wide variety of *Vibrio cholerae* strains. *Microbiol. Immunol.* 2018; 62(3):150–7. DOI: 10.1111/1348-0421.12570.

14. Islam M.T., Alam M., Boucher Y. Emergence, ecology and dispersal of the pandemic generating *Vibrio cholerae* lineage. *International Microbiology.* 2017; 20(3):106–15. DOI:10.2436/20.1501.01.291.

15. Smirnova N.I., Cheldyshova N.B., Zadnova S.P., Kutyrev V.V. Molecular-genetic peculiarities of classical biotype *Vibrio cholerae*, the etiological agent of the last outbreak Asiatic cholera in Russia. *Microb. Pathog.* 2004; 36:131–9. DOI: 10.1016/j.micpath.2003.10.004.

16. Narkevich M.I., Onischenko G.G., Lomov J.M., Moskvitina E.A., Podosinnikova L.S., Medinsky G.M. The seventh pandemic of cholera in the USSR, 1961–89. Bull. World Health Organ. 1993; 71:189–96. (Cited 10 Sept 2020). [Internet]. Available from: https://www.questia.com/library/journal/1G1-13913981/the-seventh-pandemic-of-cholera-in-the-ussr-1961-89.

17. Onishhenko G.G., Moskvitina E.A., Kruglikov V.D., Titova S.V., Adamenko O.L., Vodop'janov A.S., Vodop'janov S.O. [Epidemiological surveillance of cholera in Russia during the period of the seventh pandemic]. *Vestnik Rossiiskoi Akademii Meditsinskikh Nauk [Annals of the Russian Academy of Medical Sciences]*. 2015; 70(2):249–56. DOI: 10.15690/vramn.v70i2.1320.

18. Pisanov R., Ezhova M., Monakhova E., Cherkasov A., Krasnov Y., Vodop'yanov A., Kul'shan' T., Livanova L., Portenko S., Abdrashitova A., Kruglikov V., Titova S. [Peculiarities of genome structure of toxigenic *Vibrio cholerae* El Tor Inaba strain, isolated from a surface water body in the territory of Rostov-on-Don in 2014]. *Problemy Osobo Opasnykh Infektsii [Problems of Particularly Dangerous Infections]*. 2015; (2):63–7. DOI: 10.21055/0370-1069-2015-2-63-67.

19. Emch M., Feldacker C., Islam M.S., Ali M. Seasonality of cholera from 1974 to 2005: a review of global patterns. *Int. J. Health. Geogr*. 2008; 7:31. DOI: 10.1186/1476-072X-7-31.

20. WHO. 517 Meeting of the Strategic Advisory Group of Experts on immunization, October 2009 – conclusions and recommendations. *Wkly Epidemiol. Rep.* 2009; 84:517–32. Available from: https://www.who.int/wer/2009/wer8450/en.

21. Sarkar B.L., Kanungo S., Nair G.B. How endemic is cholera in India? *Indian. J. Med. Res.* 2012; 135:246–8. PMCID: PMC3336858.

22. Kanungo S., Sah B.K., Lopez A.L., Sung J.S., Paisley A.M., Sur D., Clemens J.D., Nair G.B. Cholera in India: an analysis of reports, 1997–2006. *Bull. World Health Organ.* 2010; 88(3):185–91. DOI: 10.2471/BLT.09.073460.

23. Ali M., Gupta S.S., Arora N., Khasnobis P., Venkatesh S., Sur D., Nair G.B., Sack D.A., Ganguly N.K. Identification of burden hotspots and risk factors for cholera in India: an observational study. *PLoS One*. 2017; 12(8):e0183100. DOI: 10.1371/journal.pone.0183100.

24. Mironova L.V., Gladkikh A.S., Ponomareva A.S., Feranchuk S.I., Bochalgin N.O., Basov E.A., Khunkheeva Z.Yu., Balakhonov S.V. Comparative genomics of Vibrio cholerae El Tor strains isolated at epidemic complications in Siberia and at the Far East. *Infect. Genet. Evol.* 2018; 60:80–8. DOI: 10.1016/j.meegid.2018.02.023.

25. Liashko V. Cholera outbreak in Donetsk Oblast, Ukraine, in 2011. *EpiNorth Journal of the Network for Communicable Disease Control in Northern and Eastern Europe*. 2012; 13:117–20. [Internet]. Available from: https://docplayer.ru/55732270-No-3-vol-journal-of-the-network-for-communicable-disease-control-in-northern-and-eastern-europe.html.

26. Domashenko O.N., Belomerya T.A., Martynova N.V., Daragan G.N., Demkovich O.O., Malakhova U.V., Zemlyanskaya G.I., Popova D.M. [Cholera in Azov area]. *Zhurnal infectologii [Journal Infectology]*. 2015; 7(2):92–7. DOI: 10.22625/2072-6732-2015-7-2-92-97.

27. Kul'shan' T.A., Krasnov Y.M., Lozovsky Y.V., Smirnova N.I. Molecular MLVA typing of typical and genetically altered natural strains of *Vibrio cholerae* El Tor biovar. *Problemy Osobo Opasnykh Infektsii [Problems of Particularly Dangerous Infections]*. 2012; (4(114)):39–43. DOI: 10.21055/0370-1069-2012-4-39-43.

28. Balakhonov S.V., Mironova L.V., Basov E.A., Gladkikh A.S., Afanasev M.V., Ganin V.S., Urbanovich L.Y., Sidorova E.A. Whole-genome sequencing of a *Vibrio cholerae* El Tor strain isolated in the imported cholera focus in Siberia. *Genome Announc*. 2015; 3(2):e01550-14. DOI: 10.1128/genomeA.01550-14.

29. Didelot X., Pang B., Zhou Z., McCann A., Ni P., Li D., Achtman M., Kan B. The role of China in the global spread of the current cholera pandemic. *PLoS Genet*. 2015; 11(3):e1005072. DOI: 10.1371/journal.pgen.1005072.

30. Almagro-Moreno S., Taylor R.K. Cholera: environmental reservoirs and impact on disease transmission. *Microbiol. Spectr.* 2013; 1(2):OH-0003-2012. DOI: 10.1128/microbiolspec.OH-0003-2012.

31. Lutz C., Erken M., Noorian P., Sun S., McDougald D. Environmental reservoirs and mechanisms of persistence of *Vibrio cholerae*. *Front. Microbiol.* 2013; 4:375. DOI: 10.3389/fmicb.2013.00375.

32. Conner J.G., Teschler J.K., Jones C.J., Yildiz F.H. Staying alive: *Vibrio cholerae's* cycle of environmental survival, transmission, and dissemination. *Microbiol. Spectr.* 2016; 4(2):10.1128/microbiolspec.VMBF-0015-2015. DOI: 10.1128/microbiolspec.VMBF-0015-2015.

33. Mavian C., Paisie T.K., Alam M.T., Browne C., Beau De Rochars V.M., Nembrini S., Cash M.N., Nelson E.J., Azarian T., Ali A., Morris J.G. Jr, Salemi M. Toxigenic *Vibrio cholerae* evolution and establishment of reservoirs in aquatic ecosystems. *Proc. Natl. Acad. Sci. USA*. 2020; 117(14):7897–904. DOI: 10.1073/pnas.1918763117.

34. Batabyal P., Mookerjee S., Palit A. Occurrence of toxigenic *Vibrio cholerae* in accessible water sources of cholera endemic foci in India. *Jpn. J. Infect. Dis*. 2012; 65(4):358–60. DOI: 10.7883/yoken.65.358.

35. Mishra A., Taneja N., Sharma M. Environmental and epidemiological surveillance of *Vibrio cholerae* in a cholera-endemic region in India with freshwater environs. *J. Appl. Microbiol*. 2012; 112(1):225–37. DOI: 10.1111/j.1365-2672.2011.05191.x.

36. Taneja N., Mishra A., Batra N., Gupta P., Mahindroo J., Mohan B. Inland cholera in freshwater environs of north India. *Vaccine*. 2019; 38(Suppl. 1):A63-A72. DOI: 10.1016/j.vaccine.2019.06.038.

37. Maramovich A.S., Urbanovich L.Ya., Kulikalova E.S., Shkaruba T.T. [The role and purpose of surface water bodies in the establishment and development of the VII cholera pandemic]. *Epidemiologiya i Infektsionnye Bolezni [Epidemiology and Infectious Diseases]*. 2009; (2):21–5.

38. Titova S.V., Monakhova E.V., Alekseeva L.P., Pisanov R.V. [Molecular genetic basis of biofilm formation as a component of *Vibrio cholerae* persistence in water reservoirs of Russian Federation]. *Ekologicheskaya Genetika [Ecological genetics]*. 2018; 16(4):23–32. DOI: 10.17816/ecogen16423-32.

39. Alam M.T., Weppelmann T.A., Longini I., DeRochars V.M.B., Morris J.G., Jr, Ali A. Increased isolation frequency of toxigenic *Vibrio cholerae* O1 from environmental monitoring sites in Haiti. *PLoS One*. 2015; 10(4):e0124098. DOI:10.1371/journal.pone.0124098.

40. Baron S., Lesne J., Moore S., Rossignol E., Rebaudet S., Gazin P., Barrais R., Magloire R., Boncy J., Piarroux R. No evidence of significant levels of toxigenic *V. cholerae* O1 in the Haitian aquatic environment during the 2012 rainy season. *PLoS Curr.* 2013; 5:ecurrents.outbreaks.7735b392bdcb749baf5812d2096d331e. DOI: 10.1371/currents.outbreaks.7735b392bdcb749baf5812d2096d331e.

41. Rebaudet S., Moore S., Rossignol E., Bogreau H., Gaudart J., Normand A.C., Laraque M.J., Adrien P., Boncy J., Piarroux R. Epidemiological and molecular forensics of cholera recurrence in Haiti. *Sci. Rep*. 2019; 9(1):1164. DOI: 10.1038/s41598-018-37706-0.

42. Moore S., Thomson N., Mutreja A., Piarroux R. Widespread epidemic cholera caused by a restricted subset of *Vibrio cholerae* clones. *Clin. Microbiol. Infect*. 2014; 20(5):373–9. DOI: 10.1111/1469-0691.12610.

43. Deen J., Mengel M.A., Clemens J.D. Epidemiology of cholera. *Vaccine*. 2020; 38(Suppl. 1):A31-A40. DOI: 10.1016/j.vaccine.2019.07.078.

44. Antonova E.S., Hammer B.K. Quorum-sensing autoinducer molecules produced by members of a multispecies biofilm promote horizontal gene transfer to *Vibrio cholerae*. *FEMS Microbiol. Lett.* 2011; 322(1):68–76. DOI: 10.1111/j.1574-6968.2011.02328.x.

45. Chawdhury F.R., Nur Z., Hassan N., von Seidlein L., Dunachie S. Pandemics, pathogenicity and changing molecular epidemiology of cholera in the era of global warming. *Ann. Clin. Microbiol. Antimicrob.* 2017; 16(1):10. DOI: 10.1186/s12941-017-0185-1.

46. Lekshmi N., Joseph I., Ramamurthy T., Thomas S. Changing facades of *Vibrio cholerae*: An enigma in the epidemiology of cholera. *Indian J. Med. Res.* 2018; 147(2):133–41. DOI: 10.4103/ijmr.IJMR_280_17.

47. Gupta S.S., Ganguly N.K. Opportunities and challenges for cholera control in India. *Vaccine.* 2020; 38(Suppl. 1):A25-A27. DOI: 10.1016/j.vaccine.2019.06.032.

48. Hu D., Yin Z., Yuan C., Yang P., Qian C., Wei Y., Zhang S., Wang Y., Yuan J., Wang M., Reeves P.R., Tu L., Chen M., Huang D., Liu B. Changing molecular epidemiology of *Vibrio cholerae* outbreaks in Shanghai, China. *mSystems*. 2019; 4(6):e00561-19. DOI: ms.00561-19.

49. Pal B.B., Khuntia H.K., Nayak S.R., Mohanty A., Biswal B. *Vibrio cholerae* O1 Ogawa strains carrying the ctxB7 allele caused a large cholera outbreak during 2014 in the tribal areas of Odisha, India. *Jpn. J. Infect. Dis.* 2017; 70(5):549–53. DOI: 10.7883/yoken.JJID.2016.585.

50. Zadnova S.P., Kul'shan' T.A., Cheldyshova N.B., Kritsky A.A., Plekhanov N.A., Smirnova N.I. Comparative analysis of survival capacity among typical and genovariant strains of *Vibrio cholerae,* biovar El Tor *in vivo* and *in vitro. Problemy Osobo Opasnykh Infektsii [Problems of Particularly Dangerous Infections]*. 2015; (4):65–9. DOI: 10.21055/0370-1069-2015-4-65-69.

51. Hasan N.A., Choi S.Y., Eppinger M., Clark P.W., Chen A., Alam M., Haley B.J., Taviani E., Hine E., Su Q., Tallon L.J., Prosper J.B., Furth K., Hoq M.M., Li H., Fraser-Liggett C.M., Cravioto A., Huq A., Ravel J., Cebula T.A., Colwell R.R. Genomic diversity of 2010 Haitian cholera outbreak strains. *Proc. Natl. Acad. Sci. USA*. 2012; 109(29):E2010-7. DOI: 10.1073/pnas.1207359109.

52. Ghosh P., Naha A., Pazhani G.P., Ramamurthy T., Mukhopadhyay A.K. Genetic traits of *Vibrio cholerae* O1 Haitian isolates that are absent in contemporary strains from Kolkata, India. *PLoS One*. 2014; 9(11):e112973. DOI: 10.1371/journal.pone.0112973.

53. Ghosh P., Kumar D., Chowdhury G., Singh P., Samanta P., Dutta S., Ramamurthy T., Sharma N.C., Sinha P., Prasad Y., Shinoda S., Mukhopadhyay A.K. Characterization of *Vibrio cholerae* O1 strains that trace the origin of Haitian-like genetic traits. *Infect. Genet. Evol*. 2017; 54:47–53. DOI: 10.1016/j.meegid.2017.06.015.

54. Ha S.-M., Chalita M., Yang S.-J., Yoon S.-H., Cho K., Seong W.K., Hong S., Kim J., Kwak H.-S., Chun J. Comparative genomic analysis of the 2016 *Vibrio cholerae* outbreak in South Korea. *Front. Public Health.* 2019; 7:228. DOI: 10.3389/fpubh.2019.00228.

55. Rashid M-U., Rashed S.M., Islam T., Johura F.T., Watanabe H., Ohnishi M., Alam M. CtxB1 outcompetes CtxB7 in *Vibrio cholerae* O1, Bangladesh. *J. Med. Microbiol.* 2016; 65(1):101–3. DOI: 10.1099/jmm.0.000190.

56. Weill F.-X., Domman D., Njamkepo E., Almesbahi A.A., Naji M., Nasher S.S., Rakesh A., Assiri A.M., Sharma N.C., Kariuki S., Pourshafie M.R., Rauzier J., Abubakar A., Carter J.Y., Wamala J.F., Seguin C., Bouchier C., Malliavin T., Bakhshi B., Abulmaali H.H.N., Kumar D., Njoroge S.M., Malik M.R., Kiiru J., Luquero F.J., Azman A.S., Ramamurthy T., Thomson N.R., Quilici M.-L. Genomic insights into the 2016–2017 cholera epidemic in Yemen. *Nature.* 2019; 565(7738):230–3. DOI: 10.1038/s41586-018-0818-3.

57. Dolores J., Satchell K.J.F. Analysis of *Vibrio cholerae* genome sequences reveals unique *rtxA* variants in environmental strains and an *rtxA* null-mutation in recent altered El Tor isolates.

mBio. 2013; 4(2):e00624-12. DOI: 10.1128/mBio.00624-12.

58. Das M.M., Bhotra T., Zala D., Singh D.V. Phenotypic and genetic characteristics of *Vibrio cholerae* O1 carrying Haitian *ctxB* and attributes of classical and El Tor biotypes isolated from Silvassa, India. *J. Med. Microbiol*. 2016; 65(8):720–8. DOI: 10.1099/jmm.0.000282.

59. Naha A., Mandal R.S., Samanta P., Saha R.N., Shaw S., Ghosh A., Chatterjee N.S., Dutta P., Okamoto K., Dutta S., Mukhopadhyay A.K. Deciphering the possible role of *ctxB7* allele on higher production of cholera toxin by Haitian variant *Vibrio cholerae* O1. *PLoS Negl. Trop. Dis*. 2020; 14(4):e0008128. DOI: 10.1371/journal.pntd.0008128.

60. Kuleshov K.V., Vodop'ianov S.O., Dedkov V.G., Markelov M.L., Kermanov A.V., Kruglikov V.D., Vodop'ianov A.S., Pisanov R.V., Chemisova O.S., Mazrukho A.B., Titova S.V., Shipulin G.A. Draft genome sequencing of *Vibrio cholerae* O1 El Tor isolates collected in the Russian Federation from imported cholera cases. *Genome Announc*. 2014; 2(4):e00624-14. DOI: 10.1128/genomeA.00624-14.

61. Smirnova N.I., Krasnov Y.M., Agafonova E.Y., Shchelkanova E.Y., Alkhova Z.V., Kutyrev V.V. Whole-genome sequencing of *Vibrio cholerae* O1 El Tor strains isolated in Ukraine (2011) and Russia (2014). *Genome Announc*. 2017; 5(8):e01640-16. DOI: 10.1128/genomeA.01640-16.

62. Katz L.S., Petkau A., Beaulaurier J., Tyler S., Antonova E.S., Turnsek M.A., Guo Y., Wang S., Paxinos E.E., Orata F., Gladney L.M., Stroika S., Folster J.P., Rowe L., Freeman M.M., Knox N., Kim B.S., Gavin H.E., Satchell K.J. Distinct roles of the repeat-containing regions and effector domains of the *Vibrio vulnificus* multifunctional-autoprocessing repeats-in-toxin (MARTX) toxin. *mBio.* 2015; 6(2):e00324–15. DOI: 10.1128/mBio.00324-15.

63. Bwire G., Sack D.A., Almeida M., Li S., Voeglein J.B., Debes A.K., Kagirita A., Buyinza A.W., Orach C.G., Stine O.C. 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.

64. Houmnanou Y.M.G., Leekitcharoenphon P., Kudirkiene E., Mdegela R.H., Hendriksen R.S., Olsen J.E., Dalsgaard A. Genomic insights into *Vibrio cholerae* O1 responsible for cholera epidemics in Tanzania between 1993 and 2017. *PLoS Negl. Trop. Dis*. 2019; 13(12):e0007934. DOI: 10.1371/journal.pntd.0007934.

65. Li F., Pang B., Yan H., Lu X., Li J., Zhou H., Cui Z., Zhao L., Mahemut, Huo D., Kan B., Jia L. Investigation of an imported cholera case in China with whole genome sequencing. *Infect. Genet. Evol.* 2020; 84:104362. DOI: 10.1016/j.meegid.2020.104362.

66. WHO. Global Task Force on Cholera Control. Ending Cholera. A Global Roadmap to 2030. 2017; 32 p. (Cited 18 June 2020). [Internet]. Available from: http://www.who.int/cholera/publications/global-roadmap.pdf.

67. Mutreja A., Dougan G. Molecular epidemiology and intercontinental spread of cholera. *Vaccine*. 2020; 38(Suppl. 1):A46–A51. DOI: 10.1016/j.vaccine.2019.07.038.

Authors:

Monakhova E.V. Rostov-on-Don Research Anti-Plague Institute. 117/40, M. Gor`kogo St., Rostov-on-Don, 344002, Russian Federation. E-mail: monakhova_ev@antiplague.ru.

Ghosh A., J.C.Bose Chair Professor of the NASI, India. ICMR-National Institute of Cholera and Enteric Diseases. P-33, CIT Scheme XM, Beliaghata, Kolkata - 700 010, India. E-mail: amitghosh24@yahoo.com.

Mutreja A. Department of Medicine, University of Cambridge. Level 5, Addenbrookes Hospital, Cambridge CB2 0QQ, United Kingdom. E-mail: am872@medschl.cam.ac.uk.

Weill F.-X. Institut Pasteur, Unité des bactéries pathogènes entériques. 28 rue du Dr Roux, 75724 Paris cedex 15, France. E-mail: francois-xavier.weill@pasteur.fr.

Ramamurthy T., INSA-Senior Scientist. ICMR-National Institute of Cholera and Enteric Diseases. P-33, CIT Scheme XM, Beliaghata, Kolkata - 700 010, India. E-mail: rama1murthy@yahoo.com.

Об авторах:

Монахова Е.В. Ростовский-на-Дону научно-исследовательский противочумный институт. Российская Федерация, 344002, Ростов-на-Дону, ул. М. Горького, 117/40. E-mail: monakhova_ev@antiplague.ru.

Ghosh A., J.C.Bose Chair Professor of the NASI, India. ICMR-National Institute of Cholera and Enteric Diseases. P-33, CIT Scheme XM, Beliaghata, Kolkata - 700 010, India. E-mail: amitghosh24@yahoo.com.

Mutreja A. Department of Medicine, University of Cambridge. Level 5, Addenbrookes Hospital, Cambridge CB2 0QQ, United Kingdom. E-mail: am872@medschl.cam.ac.uk.

Weill F.-X. Institut Pasteur, Unité des bactéries pathogènes entériques. 28 rue du Dr Roux, 75724 Paris cedex 15, France. E-mail: francois-xavier.weill@pasteur.fr.

Ramamurthy T., INSA-Senior Scientist. ICMR-National Institute of Cholera and Enteric Diseases. P-33, CIT Scheme XM, Beliaghata, Kolkata - 700 010, India. E-mail: rama1murthy@yahoo.com.