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

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

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 эпидемиям 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 настоящее время описаны геномные характеристики TVC, выделенных в России, Индии и некоторых других странах. Обзор дополнен биоинформатическим анализом генетических маркеров, значимых для дифференциации клонов, появившихся в эндемических регионах и завезенных на территории, где холера прежде отсутство-
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 (IZCB01, 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, tsunamis, 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 Hajj 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-

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les 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 (TTTGTGAT) 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\(^\text{1970-0512}\) [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, rstB3\(^\text{1993}\) for 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 ctxB3 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\(^\text{1970-0512}\) 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\(^\text{1970-0512}\) 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 58th 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\(^\text{1970-0512}\) (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
Distribution of genetic markers among *V. cholerae* strains of different origin

| Strain | Origin | Gene alleles | VSP-II | TR No. | Ref. |
|--------|--------|--------------|--------|--------|------|
| 8579 | POM02 | Taganrog-72—USSR ← Iran or India | ctaB 32 ET 1 2 x 4 pt | 4 | - |
| 8226 | KER1876614 | Kerch-74—Iran or India | ctaB 3 ET 1 2 pt | 4 | 4 |
| GP152 | CWW001 | India-79 | ctaB 3 ET 1 2 pt | 4 | 4 |
| 14450 | KER1876616 | Stavropol-90—Syria | ctaB 3 ET 1 2 pt | 4 | 4 |
| 18252 | Rostov-00 | India-2 | ctaB 3 ET 1 2 pt | 4 | - |
| PRL64 | CWW001 | India-92 | ctaB 1 ET 1 2,2 G248T | 4 | 4 |
| 1-1181 | LUC02 | Novosibir reg.-94—Turkey—Bangladesh | ctaB 1 ET 1 2,1 pt | 4 | 24 |
| 1-1187 | LUX02 | Barnaul-94—India | ctaB 1 ET 1 2 G248T | 4 | 24 |
| 1-1263 | LUP02 | Irkutsk reg.-97 ← Kazakhstan | ctaB 1 ET 1 2 G248T | Δ495-498 | 3, 24, 28, 29 |
| R1764 | JRT001 | Achinsk/Krasnoyarsk ter.-97—Kazakhstan | ctaB 1 ET 1 2 G248T | Δ495-498 | 3, 27 |
| 1-1300 | LEC02 | Sakhalin-99—China | ctaB 1 ET 1 2,1 pt | 4 | 24 |
| 16228, 17261, 17290 | Daghestan-94—Asia | ctaB 1 ET 1 2,1 pt | 4 | 3 |
| 17296 | Daghestan-94—Asia | ctaB 1 ET 1 2 G248T | 4 | 3 |
| 18329, 18336 | Kazan-01 | ctaB 1 ET 1 2 G248T | 4 | 3 |
| 18337 | Kazan-01 | ctaB 1 ET 1 2,2 G248T | 4 | 3 |
| 18847 | St.-Petersburg-05 | ctaB 1 ET 1 2,1 pt | 4 | - |
| 18367, 18368, 18369 | Rostov-01 | ctaB 1 ET 1 2 G248T | Δ495-512 | 4 | - |
| 18588 | Rostov-03 | ctaB 1 ET 1 2,1 pt | Δ502-512 | 4 | - |
| CIRS01 | CW001 | Bangladesh-02 | CIRS 1 4 2 G248T | Δ495-512 | 3, 51 |
| M1429 | AKM01 | Beloretsk Bashkortostan-94—Tajikistan | CIRS 1 4 2 G248T | C245T | Δ495-512 | 4, 27 |
| 18826 | VSO001 | Trans-05—Tajikistan | CIRS 1 4 2 G248T | C245T | Δ495-512 | 4, 27 |
| 18899 | AKM01 | Murnau-06—India | CIRS 1 4 2 G248T | C245T | Δ495-512 | 4, 9 |
| 0779 | VM001 | China-07 | CIRS 1 4 2 G248T | C245T | Δ495-512 | 4, 18 |
| 301 | JRT001 | Taganrog-11 | CIRS 3 4 2 G248T | C245T | Δ495-512 | 4, 18 |
| THSTI 31695 | KER1828927 | India-12 | CIRS 1 4 2 G248T | C245T | Δ495-512 | 4, 56 |
| 87 | JRT001, JRT002 | Rostov-14 | CIRS 1 4 2 G248T | C245T | Δ495-512 | 4, 18 |
| IDHOI 726 | WQ001 | India-09 | CIRS 7 4 2 G248T | C245T | Δ495-512 | 4, 4 |
| TSHSTI 02834 | KER188727 | India-10 | CIRS 7 4 2 G248T | C245T | Δ495-512 | 4, 4 |
| THSTI 02955 | KER188728 | India-10 | CIRS 7 4 2 G248T | C245T | Δ495-512 | 4, 4 |
| NHCC-0108 | LEC001 | Bangladesh-10 | CIRS 9 4 2 G248T | C245T | Δ495-512 | 4, - |
| 19187 | VM001 | Moscow-10—India | CIRS 7 4 2 G248T | C245T | Δ495-512 | 4, 60 |
| 19188 | VM001 | Moscow-10—India | CIRS 7 4 2 G248T | C245T | Δ495-512 | 4, 60 |
| 19191 | VM001 | Moscow-10—India | CIRS 7 4 2 G248T | C245T | Δ495-512 | 5, 60 |
| 1799 | LEB01, 1798 | SDL001 | Haiti-10 | CIRS 7 4 2 G248T | C245T | Δ495-512 | 5, 51, 52 |
| 2010A | 151 | STB01 | Haiti-11 | CIRS 7 4 2 G248T | C245T | Δ495-512 | 5, 51, 52 |
| 2012C | 301 | STB01 | Haiti-12 | CIRS 7 4 2 G248T | C245T | Δ495-512 | 5, - |
| 3 | LPH001 | Ukraine(Mariupol)-11 | CIRS 7 4 2 G248T | C245T | Δ495-512 | 4, 2, 61 |
| THSTI 26866 | KER1828925 | India-11 | CIRS 3 4 2 G248T | C245T | Δ495-512 | 5, 56 |
| THSTI 26879 | KER1828926 | India-12 | CIRS 3 4 2 G248T | C245T | Δ495-512 | 4, 56 |
| THSTI 32676 | KER1828928 | India-12 | CIRS 3 4 2 G248T | C245T | Δ495-512 | 4, 56 |
| THSTI 33102 | KER1828929 | India-12 | CIRS 4 4 2 G248T | C245T | Δ495-512 | 4, 56 |
| 6878 | NM01 | Moscow-12—India | CIRS 4 4 2 G248T | C245T | Δ495-512 | 4, 60 |
| W4-13 | VM001 | India-13 | CIRS 4 4 2 G248T | C245T | Δ495-512 | 4, - |
| THSTI 36124, 36133, 36136 | KER1828930,44-46 | India-13 | CIRS 4 4 2 G248T | C245T | Δ495-512 | 4, 56 |
| CNRVC140176 | THSTI 41049, 41055, 41081 | THSTI 26866 | India-14 | CIRS 4 4 2 G248T | C245T | Δ495-512 | 4, 56 |
| 326 | WQ001 | Moscow-14—India | CIRS 4 4 2 G248T | C245T | 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 ctaB. Genes rstr are presented as found in WGSs which not necessarily properly reflect their real distribution.
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 translational location of the toxin into eukaryotic cells [62]. Further studies on the structure of rtxA4 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 pandemics 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, and 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 IEC 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, maximal 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.

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25
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