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The risks of cholera importation and the forecasting system in epidemiological surveillance in the Russian Federation

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ABSTRACT

BACKGROUND: The risks of cholera importation into Russia are determined by the intensity of the epidemic process in endemic countries of Southeast Asia, the Eastern Mediterranean, and Africa. The assessment of risk factors for cholera importation and spread forms the basis of the risk-oriented methodology in proactive epidemiological surveillance, which ensures timely forecasting of the epidemiological situation, preparedness, and rapid response in case of its deterioration, as well as the prevention of severe consequences.

AIM: To assess the effectiveness of proactive epidemiological surveillance of cholera in Russia in 2023–2024.

MATERIALS AND METHODS: A risk-oriented approach was employed, based on the systematic collection of official data on global cholera incidence from sources such as the Weekly Epidemiological Record (WHO), official and regional WHO websites, internet resources including ProMED-mail post, ECDC, and other platforms. The study adhered to regulatory documents issued by the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing for 2023–2024 and utilized operational epidemiological analysis data from the Reference Center for Cholera Monitoring in Russia. Whole-genome sequencing (WGS) and genome assembly were conducted on the MiSeq (Illumina) platform, and genetic determinants were identified in WGS data using BioEdit 7.2.5 and BLASTN 2.2.29. Statistical data processing was performed using the StatSoft STATISTICA 6.1.478 Russian software.

RESULTS: A comprehensive analysis of global cholera incidence data was conducted, providing a realistic forecast of the risks of cholera importation into Russia in 2023. The epidemic preparedness of Rospotrebnadzor and the Ministry of Health of Russia was assessed, followed by a prompt response to two imported cholera cases detected in Tambov region and Moscow in 2023, both originating from India. Timely identification of these cases, including genomic and phylogenetic characterization of the pathogens, was achieved. Infection foci were localized and eliminated, preventing further spread. Cholera monitoring in Russia continued throughout 2024, with preventive measures implemented to minimize the risks of cholera importation and spread.

CONCLUSIONS: The analysis of the 2023–2024 monitoring results demonstrated the effectiveness of the proactive epidemiological surveillance system, which relies on early identification of cholera importation risks and their precursors, forecasting, rapid response to potential complications in epidemiological situation, and control of objects and factors contributing to infection spread. The epidemiological stability regarding cholera in Russia was ensured.

Keywords: cholera; incidence; *Vibrio cholerae* O1; phylogenetic analysis.

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Риски завоза холеры и система прогнозирования в эпидемиологическом надзоре в Российской Федерации

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АННОТАЦИЯ

Обоснование. Риски завоза холеры в Российскую Федерацию обусловлены интенсивностью эпидемического процесса по этой инфекции в эндемичных странах Юго-Восточной Азии, Восточного Средиземноморья и Африки. Оценка факторов риска завоза и распространения холеры лежит в основе методологии риск-ориентированного подхода в проактивном эпидемиологическом надзоре, который обеспечивает своевременный прогноз развития эпидситуации, готовность и оперативное реагирование в случае её осложнения, а также предотвращение тяжёлых последствий.

Цель исследования — определить эффективность проактивного эпидемиологического надзора за холерой на территории Российской Федерации в 2023–2024 годах.

Материалы и методы. Использован риск-ориентированный подход, основанный на систематизации официальных сведений о мировой заболеваемости холерой (Weekly Epidemiological Record of WHO), официальных и региональных сайтов Всемирной организации здравоохранения, интернет-ресурсов (ProMED-mail post, ECDC) и других источников. В работе руководствовались распорядительными документами Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека в 2023–2024 годах, применяли материалы оперативного эпидемиологического анализа Референс-центра по мониторингу за холерой на территории Российской Федерации. Полногеномное секвенирование и сборку геномов выполняли на платформе MiSeq (Illumina), генетические детерминанты идентифицировали в WGSs с использованием программ BioEdit 7.2.5 и BLASTN 2.2.29. Статистическую обработку полученных результатов проводили с помощью компьютерной программы StatSoft STATISTICA 6.1.478 Russian.

Результаты. Проведён анализ массива данных по заболеваемости холерой в мире, дан реалистичный прогноз вероятности рисков завоза холеры в Российскую Федерацию в 2023 году. Проверена противозэпидемическая готовность органов и организаций Роспотребнадзора и Минздрава России, осуществлено оперативное реагирование на два случая завоза холеры в Тамбовскую область и Москву из Индии в 2023 году со своевременной их расшифровкой, включая геномную и филогенетическую характеристику возбудителей. Локализованы и ликвидированы очаги инфекции, не допущено её распространения. Проведён мониторинг холеры на территории Российской Федерации в 2024 году. Осуществлены мероприятия по минимизации рисков завоза и распространения данной инфекции.

Заключение. На основе анализа результатов мониторинга 2023–2024 годов доказана эффективность системы проактивного эпидемиологического надзора, основанного на раннем выявлении рисков завоза холеры или их предвестников, прогнозировании, оперативном реагировании в случае реализации осложнения эпидситуации по холере и контроле объектов и факторов, способствующих возможному распространению инфекции. Обеспечена стабильность эпидемиологической ситуации по холере в Российской Федерации.

Ключевые слова: холера; заболеваемость; *Vibrio cholerae* 01; филогенетический анализ.

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BACKGROUND

The seventh cholera pandemic, caused by the El Tor biotype, has been ongoing for 63 years—three-quarters of the total duration of all six previous pandemics combined. In the past decade, there has been a reactivation of the epidemic process in cholera-endemic countries of Asia (India, Bangladesh, Thailand, etc.) as well as African countries (Somalia, Burundi, Zambia, Zimbabwe, Nigeria, Ethiopia, Kenya, Cameroon, the Democratic Republic of the Congo, Mozambique, and others) [1, 2]. Each year, the number of countries affected by cholera increases due to the involvement of new administrative territories and the formation of persistent endemic foci of the disease.

The assessment of risk factors for cholera importation and spread is the foundation of the risk-oriented approach [3] in proactive epidemiological surveillance, which ensures timely forecasting of the epidemiological situation, preparedness, and rapid response in case of its deterioration, as well as the prevention of severe consequences.

The forecasting system within proactive cholera surveillance is currently based on a multifactorial analysis of data on the global cholera epidemic situation and on realistic forecasts specifying the spatial and temporal configuration of importation risks to Russia at present and in the near future.

AIM

The work aimed to assess the effectiveness of the risk-oriented approach in proactive epidemiological surveillance of cholera in the Russian Federation in 2023–2024.

METHODS

A risk-oriented approach [3] was employed, based on the systematization of official data on global cholera incidence (Weekly Epidemiological Record of WHO), official and regional WHO websites, internet resources (ProMED-mail post, ECDC), and other sources. The study was conducted in accordance with the regulatory documents of the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing for 2023–2024 and made use of operational epidemiological analysis materials from the Reference Center for Cholera Monitoring in the Russian Federation. Cholera surveillance in the Russian Federation was conducted in compliance with Sanitary and Epidemiological Rules and Regulations SanPiN 3.3686-21, paragraph 1929. Indication and identification of isolated cholera *Vibrio* strains were performed in accordance with Methodological Guidelines MU 4.2.3745-22, MU 4.2.3746-22, MU 4.2.4062-24 (Amendment No. 1 to MU 4.2.3746-22), and MU 4.2.4030-24 (Amendment No. 1 to MU 4.2.3745-22) [4–7]. A weekly multifactorial analysis of a wide range of information sources was conducted, including data on incidence, contributing factors and conditions, and various emergency situations that could facilitate cholera activation.

Whole-genome sequencing and genome assembly were performed using the MiSeq (Illumina) platform, as previously described [8]. Genetic determinants were identified in WGS data using BioEdit 7.2.5 (<http://www.mbio.ncsu.edu/bioedit>) and BLASTN 2.2.29 (<http://blast.ncbi.nlm.nih.gov>). Statistical data processing of the obtained results was carried out using StatSoft STATISTICA 6.1.478 Russian software.

RESULTS

A multifactorial analysis of cholera incidence revealed that between 2014 and 2023, cholera was reported in 87 countries and 500 administrative territories, of which 94 are considered endemic [1, 2]. In 2023, 38 countries reported more than 856,000 cholera cases. Asian countries continued to account for the largest proportion of global incidence, comprising 73% of total cholera cases. In 2023, cholera affected 47 countries worldwide, 11 of which were endemic (see Fig. 1).

It was established that the intensity of the epidemic process in cholera-endemic countries, along with the spread of infection to certain non-endemic territories, in combination with accompanying socio-economic factors and conditions, created a high potential risk of cholera importation into the Russian Federation in 2023.

Based on this and in accordance with Resolution No. 7 of the Chief State Sanitary Physician of the Russian Federation dated May 24, 2023, On Additional Measures for Cholera Prevention in the Russian Federation [9], an assessment was conducted of hospital facilities (over 550) and laboratories (over 640) across Russia to ensure the preparedness of all 89 constituent entities of the Russian Federation for preventive and anti-epidemic measures against cholera (see Fig. 2).

It was found that from 1998 to 2023, epidemic manifestations of cholera in Russia were limited to isolated imported cases with no further transmission (see Table 1).

The projected risks for 2023 were realized in July and September, when two cases of cholera importation from India were registered.

In June 2023, the first imported cholera case in the country was recorded in a citizen of India who arrived from Delhi to Moscow on a flight carrying 249 passengers. The second importation occurred in September 2023 and was detected in a Russian citizen who had traveled on a business trip to South Asian countries endemic for cholera (India, Bangladesh, Indonesia) [10, 11]. According to the comprehensive five-year plan of preventive and anti-epidemic measures for the sanitary protection of the territory, a set of anti-epidemic measures was initiated. Within 24 hours after the clinical sample was delivered to the laboratory, indication, identification, and genomic and phylogenetic characterization of the isolated *Vibrio cholerae* strains were performed in accordance with the algorithm of implementation and further development prospects (see Fig. 3).

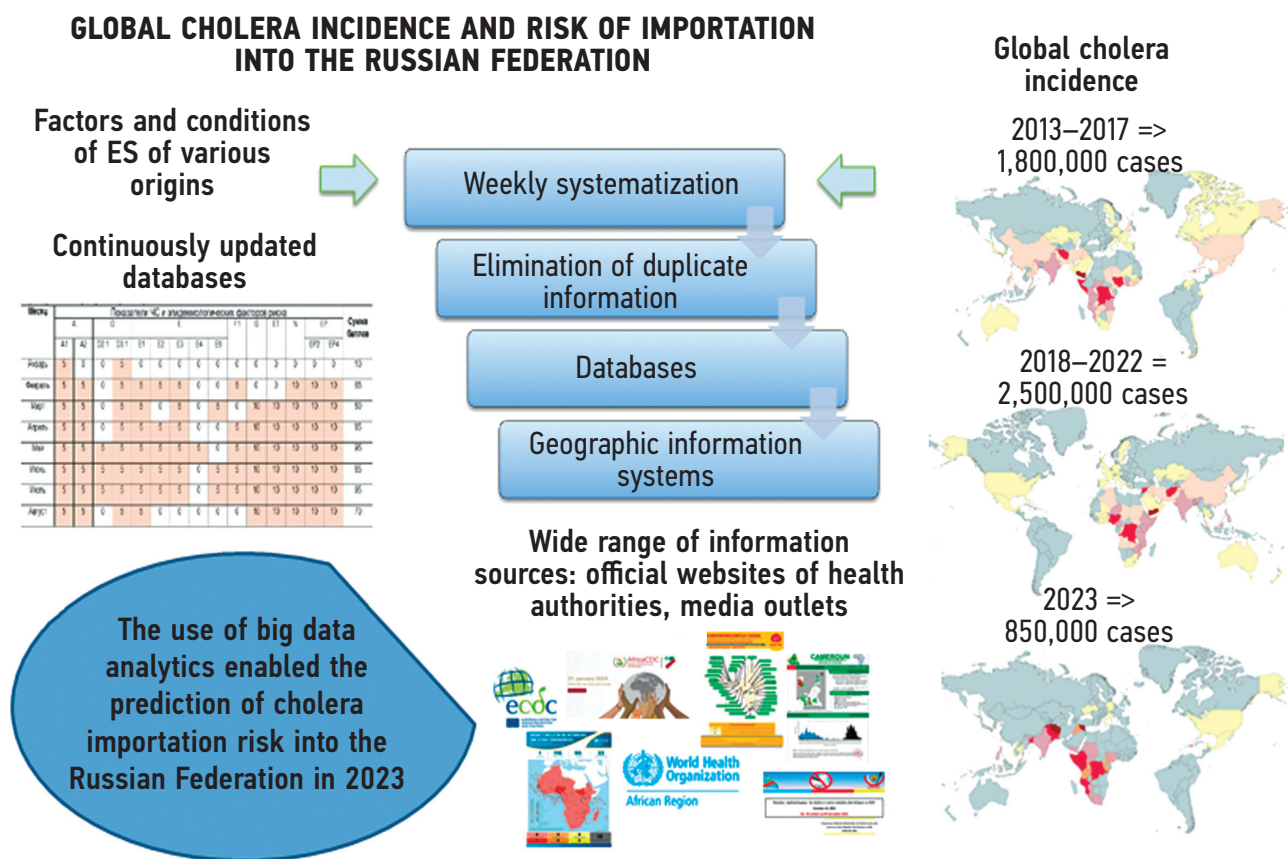


Fig. 1. Multifactor information monitoring of biological threats related to cholera. ES, emergency situation.

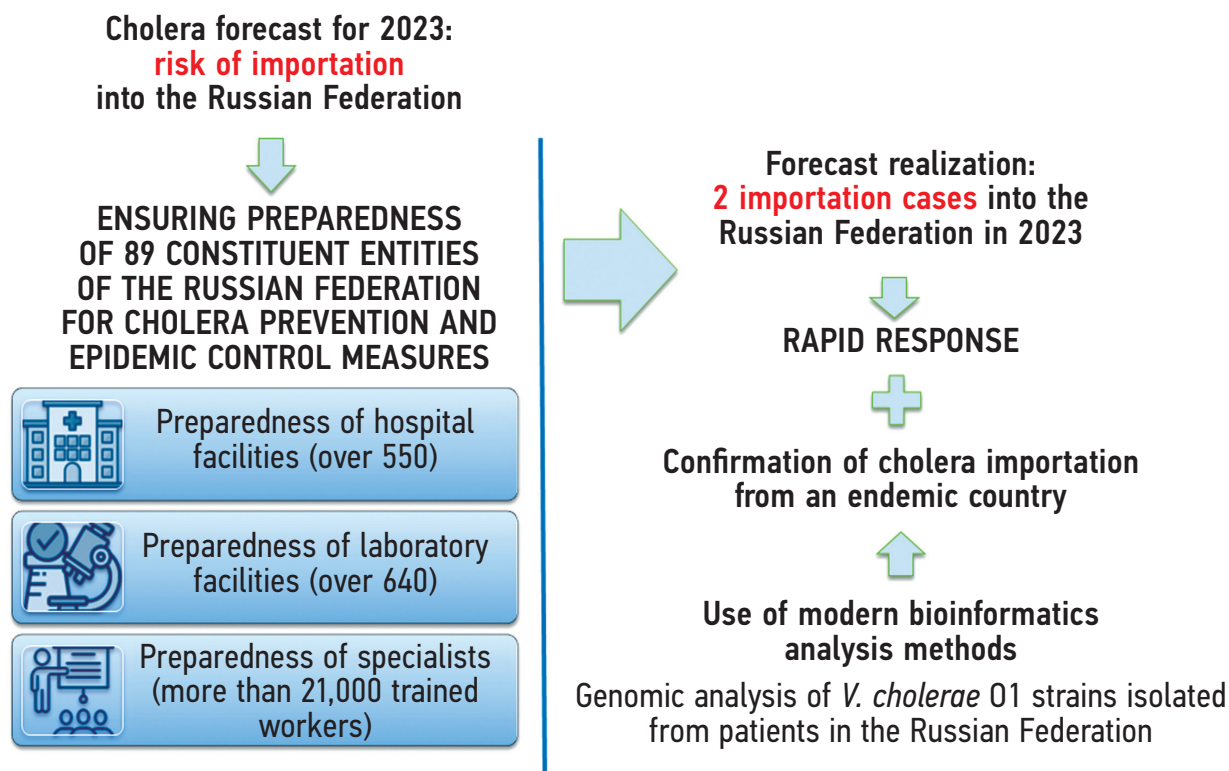


Fig. 2. Cholera forecast, risk realization, and counteraction to biological threats in the Russian Federation.

Table 1. Importation of cholera cases (without further transmission) into the Russian Federation, 1998–2023

Strain designation	Original strain ID	Year, country of origin, and location of importation
<i>V. cholerae</i> El Tor Ogawa	76A198	1998, from India to Omsk
<i>V. cholerae</i> El Tor Inaba	1	2004, from India to Beloretsk, Republic of Bashkortostan
<i>V. cholerae</i> El Tor Inaba	1	2005, from Tajikistan to Konakovo, Tver Region
<i>V. cholerae</i> El Tor Inaba	K-35	2005, from Tajikistan to Moscow
<i>V. cholerae</i> El Tor Inaba	3	32006, from India to Murmansk
<i>V. cholerae</i> El Tor Ogawa	L-4150	2010, from India to Moscow
<i>V. cholerae</i> El Tor Ogawa	89	2010, from India to Moscow
<i>V. cholerae</i> El Tor Ogawa	86	2010, from India to Moscow
<i>V. cholerae</i> El Tor Ogawa	6878	2012, from India to Moscow
<i>V. cholerae</i> El Tor Ogawa	3265/80	2014, from India to Moscow
<i>V. cholerae</i> El Tor Ogawa	5342	2023, from India to Moscow
<i>V. cholerae</i> El Tor Ogawa	6	2023, from India via Moscow to Rasskazovo, Tambov Region

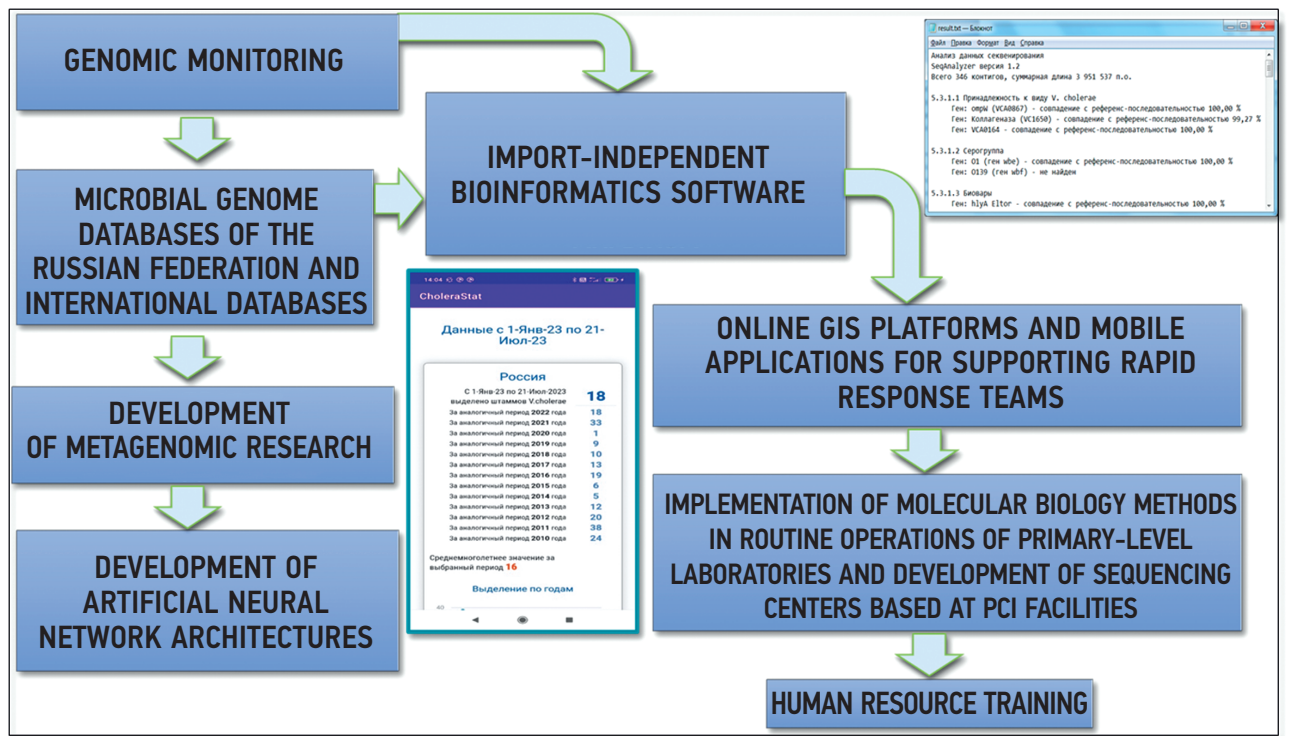


Fig. 3. Genomic monitoring, bioinformatics and IT technologies in the activities of the Reference Center for Cholera Monitoring in the Russian Federation. PCI, Plague Control Institution.

The analysis of these importation cases revealed a very few number of patients but numerous contacts. Along the travel route, at the place of residence and work, and among medical personnel, a total of 553 contacts were examined. Two patients were confirmed with isolation of toxigenic *Vibrio cholerae* O1 strains, two asymptomatic carriers of

a non-toxigenic *Vibrio cholerae* O1 serogroup strain were identified, and 17 cases involved the isolation of non-toxigenic *Vibrio cholerae* non-O1/non-O139 strains [10].

Genomic monitoring enabled the timely detection of toxigenic *Vibrio cholerae* strains atypical for non-endemic regions of Russia. The strains isolated from patients who arrived from India

in 2023, passing through Moscow to the city of Rasskazovo in the Tambov Region, belonged to genotype *ctxB7* [10]. Different gene variants of toxigenic *Vibrio cholerae* are characterized by combinations of distinct genetic markers (see Table 2).

According to the forecast of the Reference Center for Cholera Monitoring regarding the probability of *Vibrio cholerae* detection in water bodies of the Russian Federation in 2023, monitoring of contamination of surface water sources and other environmental objects with *Vibrio cholerae* O1 and O139 serogroups was launched in the Russian regions in April, upon reaching a surface water temperature of 14°C.

In the course of cholera epidemiological surveillance in 2023, a total of 52 non-toxigenic *Vibrio cholerae* O1 serogroup strains were isolated from environmental water samples across 11 constituent entities of the Russian Federation.

The 2023 strains clustered according to genetic similarity, grouped with strains previously isolated from surface water bodies at various times and in different regions of Russia, indicating the existence of a heterogeneous population of these microorganisms in the country and the absence of new introductions (see Fig. 4).

To determine the association between surface water temperature and *Vibrio cholerae* isolation, a formal request was sent to the heads of Rospotrebnadzor offices in the constituent entities of the Russian Federation. A retrospective analysis of the data collected over a five-year period (2019–2023) revealed that *Vibrio cholerae* can be isolated even when water temperatures are below 15°C.

The systematization and differentiation of cholera morbidity data on a weekly (and at times daily) basis continued into 2024. It was found that, despite a smaller number of affected countries (42), the number of endemic countries increased to 18, with more than 470,000 cholera cases reported [12–14]. Migration patterns have been influenced by modern transportation links (primarily air travel) between cholera-endemic and cholera-free countries, increasing the risk of importation of the infection within a time frame shorter than the cholera incubation period, including into Russia.

In 2024, according to the directive by Rospotrebnadzor as of March 11, 2024, On the Implementation of Measures for

Cholera Prevention, monitoring of contamination with *Vibrio cholerae* O1 and O139 serogroups in surface water bodies and other environmental objects began on April 15 in cities of the following categories: 1) cities with a permanent population over 2 million; 2) cities with a permanent population over 1 million; 3) cities with a seaport (navigable waterways); 4) resort cities; 5) territories of the new constituent entities of the Russian Federation.

The next stage of proactive epidemiological surveillance for cholera was the development of a system for the prompt assessment of the cholera epidemiological situation in any constituent entity of the Russian Federation. This led to the implementation of a weekly data request (cholera report) pursuant to the letter issued by Rospotrebnadzor on April 2, 2024, titled “On the Implementation of Cholera Prevention Measures.” The report includes 11 tables that provide a comprehensive overview of the cholera epidemiological situation in each constituent entity of the Russian Federation. The report enables the monitoring of migration activity of incoming individuals, which is one of the key drivers of outbreak development worldwide. Over the first three quarters of 2024, more than 62,000 individuals arrived in 30 constituent entities of Russia, of whom 26.8% (16,866 individuals) came from cholera-affected countries. To ensure early detection of cholera cases and carriers, 4434 tests for cholera were conducted using samples from operational wastewater treatment plants prior to treatment, and 7489 tests were conducted after treatment in 43 constituent entities of the Russian Federation. In 2024, one *Vibrio cholerae* O1 strain was isolated from untreated sewage in the Republic of Kalmykia, and another *Vibrio cholerae* O1 strain was isolated from seawater at a wastewater discharge site in the Primorsky Territory.

During cholera epidemiological monitoring in 2024, a total of 337 *Vibrio cholerae* O1 strains were isolated from environmental surface water samples, including 335 non-toxigenic and 2 toxigenic strains. These two strains were isolated from the Temernik River in Rostov Region in the context of a stable cholera epidemiological situation. All strains from this clonal complex belong to the first group

Table 2. Combination of genetic markers characteristic of different genovariants of toxigenic *Vibrio cholerae*

Genovariant	Genetic marker				
	<i>ctxB</i>	<i>tcpA</i>	<i>rtxA</i>	VSP-II	<i>rtxC</i>
Typical El Tor	3	ET	1	pt	+
Early genovariants	1	ET	1	pt	+
Pre-Haitian	1	CIRS	4	Δ495-512	+
Haitian	7	CIRS	4	Δ495-512	+
Post-Haitian	7	CIRS	4a	Δ495-512	+
Classical	1	class.	–	–	–

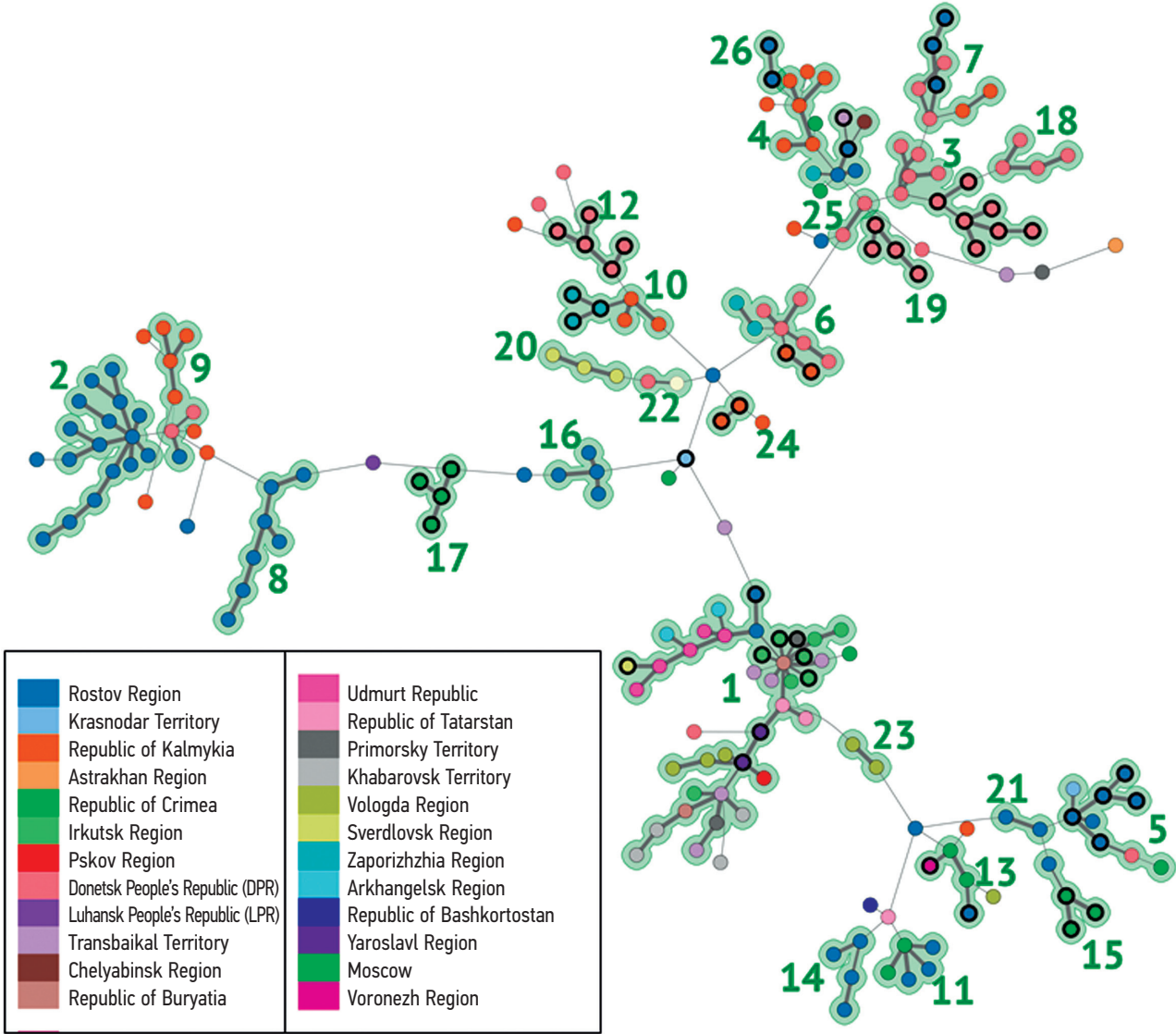


Fig. 4. Dendrogram based on SNP analysis of whole-genome sequences of non-toxicogenic *Vibrio cholerae* O1 strains isolated from surface water bodies in the constituent entities of the Russian Federation between 2005 and 2023. Strains isolated in 2023 are marked with bold outlines.

of genetic variants with the *ctxB1* genotype, which has a reduced epidemic potential. The obtained data analysis is ongoing.

Additional cholera prevention recommendations issued on July 1, 2024 (No. 02/11115-2024-32) and August 1, 2024 (No. 02/13275-2024-27) enabled the control of environmental objects and factors. Upon isolation of *Vibrio cholerae* from surface water sources, Rospotrebnadzor specialists conducted an epidemiological investigation, collected water samples until three consecutive negative results were obtained, and performed sanitary-microbiological testing. During an epidemiological investigation in Rostov Region, unauthorized discharge of liquid of unknown origin from private household pipes into the Temernik River was detected. In 2024, two toxigenic *Vibrio cholerae* strains of the O1 serogroup were isolated from the surface waters of the Temernik River in

the absence of any epidemic manifestations of cholera. This confirms the possibility of waterborne transmission of the infection.

Between calendar weeks 19 and 46 of 2024, three peaks in the isolation of *Vibrio cholerae* O1 from surface water bodies were recorded (see Fig. 5). Additionally, as forecasted, during 2023–2024, non-agglutinating vibrios (NAG-vibrios) were isolated from patients with acute intestinal infections, mainly residents of the southern regions of the country (Donetsk People's Republic, Rostov, and Zaporizhzhia Regions).

DISCUSSION

In 2023–2024, the global cholera situation remained unfavorable, with a high risk of importation into non-endemic territories. Relevant factors contributing to the intensification

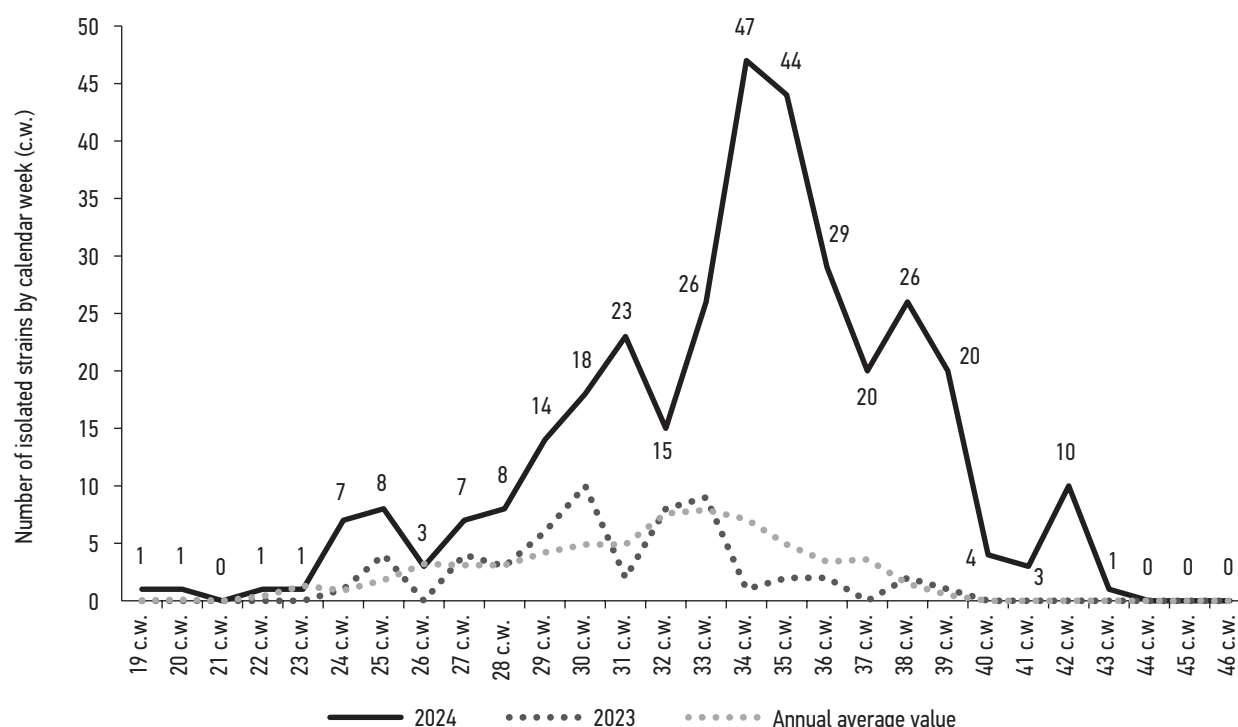


Fig. 5. Comparison of *Vibrio cholerae* O1 strain isolation by calendar weeks in the Russian Federation. The annual average represents data over a 13-year period.

of the epidemic process in many countries continued to include natural, social, and political emergencies, which resulted in disruptions of water supply and sanitation systems, limited population access to safe drinking water, medical care, and other essential services. These emergencies also caused forced migration of populations affected by such emergencies and, as a consequence, high population density in temporary accommodation sites. Additionally, national traditions and poor sanitation practices in certain countries had a significant impact on the cholera epidemiological situation [12].

The results of cross-check inspections proved to be an effective measure in assessing the preparedness of hospital and laboratories for implementing measures to prevent the importation and spread of cholera epidemics in the Russian Federation in 2023 [11].

The attribution of *Vibrio cholerae* strains isolated from cholera patients to the post-Haitian group indicated their imported nature (see Table 1). These post-Haitian strains are still circulating in India, and clinical isolates of these genetic variants predominated in border regions of Bangladesh in 2021–2022. No connection was observed between the 2023 strains and the clinical strains imported from India to Moscow in 2010, 2012, and 2014 [11].

The risks of cholera importation and spread are assessed through monitoring studies of environmental samples (from surface water bodies and wastewater) aimed at the timely detection of toxigenic *Vibrio cholerae* strains.

In 2023, an unconfirmed importation of the infection was identified due to the isolation of a toxigenic *Vibrio cholerae*

strain from a water body. This strain belonged to a group not currently prone to widespread epidemic transmission. The strain carried a genotype that had emerged in the 1990s and was classified as a pre-Haitian *ctxB1* variant (see Table 2). The detection of a strain with this genotype in Rostov-on-Don in 2023 may indicate an undetected importation from abroad without further transmission and suggests that such isolates are still circulating in certain endemic areas of Asian countries [15].

The detection of non-toxigenic *Vibrio cholerae* O1 serogroup strains and NAG vibrios in surface water at lower temperatures (ranging from 19.8 ± 0.9 to $25.2 \pm 1.1^\circ\text{C}$) [16, 17] serves as an indicator of environmental conditions that may be favorable for *Vibrio cholerae* O1 and confirms the pathogen's ability to survive under the given aquatic conditions. It also points to the potential for waterborne transmission of cholera in the event that a toxigenic strain enters the water body. These strains exhibited diverse sets of virulence factor determinants, which under certain conditions may potentially cause sporadic cases or outbreaks with clinical manifestations of gastroenteritis due to the expression of genetic determinants of a wide range of virulence factors [18]. The number of *Vibrio cholerae* O1 strains isolated from water sources in 2024 exceeded the number recorded in 2023 by a factor of 5.8. Genotypic characterization of these strains is ongoing.

The results of the current year's cholera monitoring studies continue to be analyzed. The forecast for 2024 [18] has so far been confirmed. Although no cholera importations have been registered in the Russian Federation in 2024 to date, the

risk of epidemic complications remains. As predicted, in the absence of identified patients and cholera carriers, sporadic detections of toxigenic *Vibrio cholerae* O1 serogroup strains were recorded in water samples, resulting from unconfirmed importations from cholera-affected areas.

The isolation of NAG vibrios from patients with acute intestinal infections indicates the presence of risk-forming factors and conditions that facilitate human contact with environmental objects contaminated by *Vibrio cholerae*.

CONCLUSION

The risk-based proactive epidemiological surveillance of cholera in the Russian Federation, including genomic monitoring, proved effective and enabled the forecasting, detection, and containment of cholera importations from India in 2023; identification of an unconfirmed introduction of the infection through the isolation of a toxigenic *Vibrio cholerae* strain from a water body, belonging to a group of strains currently not prone to widespread epidemic dissemination; confirmation that the nontoxigenic *Vibrio cholerae* O1 strains isolated from surface water bodies in the Russian Federation in 2023 had been previously detected at different times and in various territories of the country, were not of imported origin, and have been circulating for extended periods in surface water bodies of constituent entities of the Russian Federation—indicating a risk of waterborne transmission in the event of the introduction of

a toxigenic strain; and provision of a forecast for the cholera epidemiological situation in the Russian Federation for 2024, which is currently being confirmed.

Thus, the timely identification of cholera importation risks and the forecasting system ensured a rapid response to confirmed importations, as well as the stability of the cholera epidemiological situation in the Russian Federation during 2023–2024, which confirms the properly organized sequence of actions and the effectiveness of proactive epidemiological surveillance of cholera in the Russian Federation.

ADDITIONAL INFORMATION

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Authors' contribution. All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work. I.V. Savina: data collection and analysis, writing and editing of the article text; V.D. Kruglikov: concept development, data analysis, writing and editing of the article; N.E. Gaevskaya: concept development, data analysis, editing of the article; E.V. Monakhova: data analysis, editing of the article section; A.S. Vodopyanov: data analysis; A.K. Noskov: concept development, application of risk-based approach in proactive epidemiological analysis for cholera.

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