



## ORIGINAL STUDY ARTICLE

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## Genetic characteristics of adenoviruses (*Adenoviridae: Mastadenovirus*) circulating among military personnel with respiratory infections during the 2023–2024 season in the Sverdlovsk Region

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### Abstract

The relevance of the conducted study is determined by the high epidemiological significance of adenovirus in the structure of acute respiratory viral infections (ARVI), particularly among military personnel. Previously, adenovirus was identified in 90% of cases of patients with community-acquired pneumonia hospitalised from organised military groups, leading to significant lung damage, various disabling complications, and possible fatal outcomes.

**The aim of this study** was to investigate the genetic diversity of adenoviruses detected in patients with respiratory infections among military personnel during the 2023–2024 influenza and ARVI epidemic season in the Sverdlovsk Region.

**Materials and methods.** Sanger sequencing was performed for 79 clinical samples (nasopharyngeal swabs) that tested positive for adenovirus. The samples were collected from military personnel exhibiting symptoms of ARVI who were receiving treatment in military medical institutions in the Sverdlovsk Region during the 2023–2024 influenza and ARVI epidemic season (October to February).

**Results.** Six genetic variants of adenovirus were identified: B3, B7, B14, B55, C2, and C5, with genotype B55 being predominant and associated with severe and moderately severe disease.

**Conclusion.** The study provides insight into the genetic diversity of adenoviruses circulating among military personnel in the Sverdlovsk Region during the 2023–2024 epidemic season. Genetic surveillance of circulating adenoviruses is essential for developing specific preventive measures, improving non-specific prevention methods, and identifying optimal drugs for aetiologic therapy.

**Keywords:** adenovirus; military personnel; genovariants; pneumonia; B55

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**Conflict of interest.** The authors declare no apparent or potential conflicts of interest related to the publication of this article.

**Ethics approval.** The study was conducted with the informed consent of the patients. The research protocol was approved by the Ethics Committee of the FSRIVI «Virome» Rospotrebnadzor (Protocol No. 4 from August 9, 2022).

ОРИГИНАЛЬНОЕ ИССЛЕДОВАНИЕ

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# Генетическая характеристика аденовирусов (*Adenoviridae: Mastadenovirus*), циркулирующих среди больных респираторными инфекциями военнослужащих в эпидемиологический сезон 2023–2024 годов в Свердловской области

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## Резюме

**Актуальность** проведенного исследования определяется высокой эпидемиологической значимостью аденовируса в структуре острых респираторных вирусных инфекций (ОРВИ), особенно среди военнослужащих. Установлено, что в 90% случаев у госпитализированных из организованных военных коллективов пациентов с внебольничной пневмонией идентифицировался аденовирус, вызывающий большой объем поражения легких, различные инвалидизирующие осложнения, возможные летальные исходы.

**Цель исследования:** изучение генетического разнообразия аденовирусов, обнаруженных у больных респираторными инфекциями военнослужащих в эпидемиологический сезон гриппа и ОРВИ 2023–2024 гг. в Свердловской области.

**Материалы и методы.** Проведено исследование 79 образцов клинического материала (назофарингеальные мазки), положительного на наличие аденовируса, с помощью метода фрагментного секвенирования по Сэнгеру. Материал был собран от военнослужащих с симптомами ОРВИ, находящихся на лечении в военно-медицинских организациях Свердловской области в период эпидсезона гриппа и ОРВИ 2023–2024 гг. (октябрь–февраль).

**Результаты.** Выявлено 6 геновариантов аденовируса: В3, В7, В14, В55, С2 и С5, при этом в большей степени определялся генотип В55, вызывающий тяжелое и средней степени тяжести течение болезни.

**Заключение.** Проведенное исследование дает представление о генетическом разнообразии аденовирусов, циркулирующих среди военнослужащих на территории Свердловской области в эпидсезоне 2023–2024 гг. Изучение генетических вариантов циркулирующих аденовирусов необходимо для разработки средств специфической профилактики заболевания, совершенствования методов неспецифической профилактики, поиска оптимальных препаратов для этиотропной терапии.

**Ключевые слова:** аденовирус; военнослужащие; геноварианты; пневмония; В55

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**Финансирование.** Авторы заявляют об отсутствии внешнего финансирования при проведении исследования. Исследование выполнено за счет государственного бюджета в рамках федерального проекта «Санитарный щит – безопасность для здоровья (предупреждение, выявление, реагирование)», а также в рамках выполнения научно-исследовательской работы «Интегрированный подход к изучению эпидемиологических и молекулярно-генетических особенностей гриппа и ОРВИ при тяжелых клинических формах в период массовой вакцинопрофилактики» (рег. № 12104150044–2).

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

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## Introduction

Despite advances in the diagnosis and treatment of acute respiratory viral infections (ARVI), adenovirus infection remains a significant socio-economic, medical, and epidemiological concern for the Armed Forces of the Russian Federation. This is due to the lack of specific preventive measures, the high proportion of adenovirus infection (64.6%) in the etiological structure of ARVI among military personnel, the tendency toward a protracted and recurrent course of the disease, and the frequent development of bronchopulmonary complications [3].

Human adenoviruses of the genus *Mastadenovirus* are classified into seven groups (A–G) based on the degree of DNA homology and the GC base pair content [4]. Different serotypes exhibit varying tissue tropism, which correlates with the clinical manifestations of the infection [5]. Currently, there are 51 serotypes and 113 genotypes of adenoviruses [6]. Respiratory pathogens include groups B (B3, B7, B14, B16, B21, and B55), C (C1, C2, C5, C6, and C57), and E (E4). Previous laboratory studies on the genotyping of clinical material from military personnel in various countries with adenovirus infection have identified the circulation of E4 [4], B4, and B7 [7–9], B14 [9, 10], B (P14H11F14) [11], and B55 [4, 12–15].

In recent years, phylogenetic analysis has increasingly identified new serotypes or subtypes that arise as a result of genomic recombination between hexon, fiber, and penton regions [16]. Thus, genetic variants that appeared after the 52nd variant in groups A, B, and C are almost all recombinants [17]. The most prominent representative of this group is adenovirus B55. A complete genomic analysis showed that genotype B55 is a Trojan horse that contains a recombinant genome comprising both the renal pathogen B11, which provides an antigenic epitope, and the respiratory pathogen B14, which determines cellular tropism, biological, and pathogenic properties [18, 19]. This virus likely emerged as a result of an outbreak of acute respiratory infection at a military training base (Spain, 1969) [20], and subsequently appeared periodically in Turkey (2004) and Singapore (2005); in China, it first caused disease in Shanxi Province in 2006 and was mistakenly identified as type 11a [12, 14, 18, 19]. Between May and June 2014, an outbreak of respiratory infection was reported at a military training center in Shandong Province, China; the pathogen was adenovirus type B (P14H11F14), phylogenetically very similar to adenovirus B55 isolated from the general population in China. Adenovirus B (P14H11F14) was characterized as less virulent than B55 and caused a mild course of the disease [11]. However, only isolated cases of this genetic variant were reported, and it was subsequently replaced by adenovirus B55. Based on the results of their study, Korean scientists determined that a major outbreak of adenovirus infection, which began in 2014, is currently ongoing within the Korean armed forces, and that the B55 genetic variant is the predominant strain [12, 13].

According to the literature data, the adenovirus genetic variant B55 has now spread to many countries, evading

herd immunity, causing severe disease, and leading to outbreaks [10, 12–14, 16, 19, 21].

The aim of this study was to investigate the genetic diversity of adenoviruses detected in military personnel with respiratory infections during the 2023–2024 influenza and ARVI epidemic season in the Sverdlovsk Region.

## Materials and methods

A study was conducted on 397 clinical samples (nasopharyngeal swabs) from military personnel with symptoms of ARVI who were receiving treatment at military medical facilities in the Sverdlovsk Region. The samples were collected during the 2023–2024 influenza and ARVI epidemic season (October–February).

The study was conducted with the informed consent of the patients. The research protocol was approved by the Ethics Committee of the FSRIVI «Virome» Rosпотребнадзор (protocol № 4 from 09.08.2022).

The study was conducted using the polymerase chain reaction (PCR) method with the AmpliSens ARVI-Screen-FL test system (Central Research Institute for Epidemiology, Russia) and Real-time CFX96 Touch thermocycler (Bio-Rad Laboratories, USA). Analysis of genetic variations in the detected adenoviruses was performed using the Applied Biosystems 3500 Series Genetic Analyzer (Thermo Fisher Scientific, USA) via fragment sequencing of the hexon gene, using primers developed by X. Wu et al. [17]. The Unipro UGENE program was used for bioinformatics processing of the sequences. The obtained data were uploaded to the VGARus information platform. Statistical analysis of the study results was performed using Microsoft Excel 2010 and Past 4.10. Qualitative characteristics are presented as proportions. To characterize the accuracy coefficient of the indicators (proportions, intensity index), the margin of error ( $\pm m$ ) was used; differences were considered statistically significant at a 95% confidence interval ( $p < 0.05$ ) [22].

## Results

PCR testing of 397 clinical samples from patients with moderate to severe symptoms of ARVI revealed that 156 (39.3%) samples tested positive for respiratory viruses. Adenoviruses were detected in  $53.2 \pm 6.6\%$  of positive samples (83 samples); rhinoviruses (41%) and seasonal coronaviruses (12.8%) were present in a smaller proportion; and parainfluenza viruses (5.1%) and respiratory syncytial viruses (0.6%) were detected in isolated samples (Fig. 1).

Adenovirus was detected in nasopharyngeal swabs from 42 (50.6%) patients with pneumonia, suggesting its role as an etiological agent or as a cofactor in the development of the disease. To definitively confirm the etiology, testing of lower respiratory tract specimens and the exclusion of bacterial pathogens are necessary. Adenovirus was also detected in patients with ARVI—31 cases (37.3%), 4 cases (4.8%) of rhinotracheitis, 3 cases (3.6%) of pharyngitis, 2 cases (2.4%) of bronchitis, and 1 case (1.3%) of intestinal infection (Fig. 2).

In 20 (24%) cases, adenovirus infection was accompanied by at least one other respiratory pathogen. The most common co-infections were adenovirus with rhinovirus (14 positive samples), with seasonal coronavirus (6), and with seasonal coronavirus and parainfluenza (1). At the same time, the combination of adenovirus with rhinovirus caused milder forms of the disease (ARVI, pharyngi-

tis, rhinotracheitis), while the combination with seasonal coronavirus led to the development of pneumonia.

It should be noted that the absence of bacteriological studies and material from the lower respiratory tract does not allow for a complete differentiation between viral and viral-bacterial pneumonia.

Of the 83 samples that tested positive for adenovirus by PCR, 79 were successfully genotyped using fragment sequencing of the hexon gene, resulting in the identification of six adenovirus genotypes: B3, B7, B14, B55, C2, and C5. The most frequently detected variants were B55 (39 samples; 49.4%) and B3 (26; 32.9%), while C2 (9; 11.4%), B7 (2; 2.5%), C5 (2; 2.5%), and B14 (1; 1.3%) were less common (Fig. 3).

It was noted that genetic variant B55 was identified as the main etiological agent of pneumonia, B3 and B55 – of acute respiratory viral infection (ARVI), C2 – of rhinotracheitis, C2 and B3 – of pharyngitis, B3 – of acute bronchitis, and B14 – of intestinal infection (Fig. 4).

It was found that at the beginning of the 2023–2024 influenza and ARVI season (October, November), genetic variants B3 and B55 were the predominant circulating strains, followed to a lesser extent by C2 and C5; in January and February, adenovirus B55 was detected in patient specimens.

According to phylogenetic analysis, certain adenoviruses were most closely related to strains from the United States (45.6%), China (43%), and Japan (11.4%) (Fig. 5).

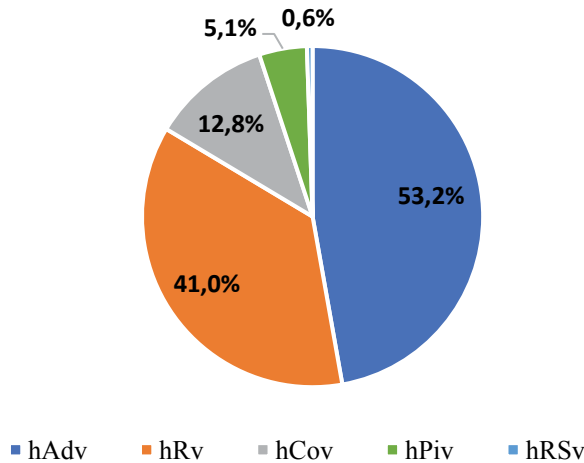


Fig. 1. The etiological structure of ARVI in military personnel in the 2023–2024 epidemic season.

Рис. 1. Этиологическая структура ОРВИ у военнослужащих в эпидсезон 2023–2024 гг.

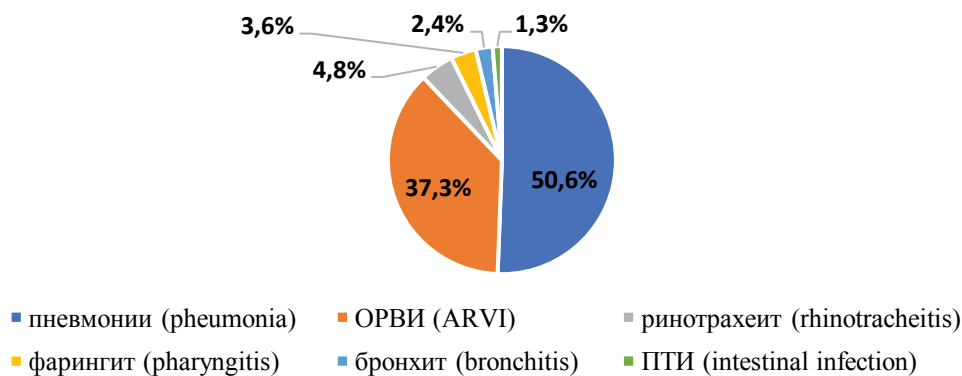


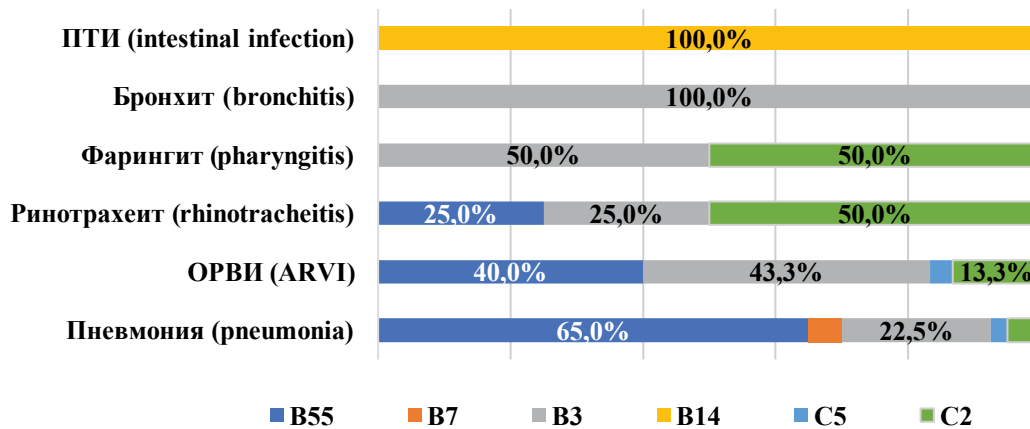
Fig. 2. Structure of clinical forms of adenovirus infection in military personnel in the 2023–2024 epidemic season

Рис. 2. Структура клинических форм аденовирусной инфекции у военнослужащих в эпидсезон 2023–2024 гг.



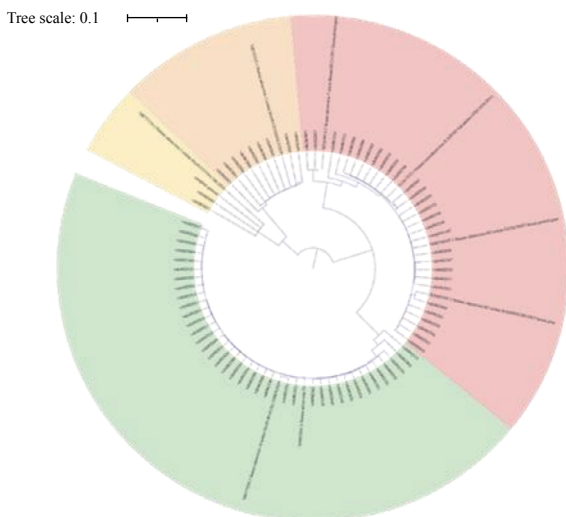
Fig. 3. Adenovirus diversity in military personnel based on fragmentary sequencing of hexon gene (2023–2024).

Рис. 3. Разнообразие аденовирусов у военнослужащих по результатам фрагментного секвенирования гена hexon (2023–2024 гг.).



**Fig. 4.** The role of gene variants in the development of clinical forms of adenovirus infection in military personnel (2023–2024).

**Рис. 4.** Роль геновариантов в развитии клинических форм аденовирусной инфекции у военнослужащих (2023–2024 гг.).



**Fig. 5.** Hexon gene phylogenetic tree of human adenovirus.

**Рис. 5.** Филогенетическое дерево по гену гексон аденовируса человека.

### Discussion

Adenovirus is one of the key pathogens of ARVI among military personnel. Previous molecular genetic studies of adenovirus genotypes circulating among military personnel in the Russian Federation do not provide a complete picture of the genetic diversity of this virus. This study identified the circulation in the Sverdlovsk Region of an epidemiologically significant adenovirus genotype – B55 – which affects the lower respiratory tract and causes severe disease. This viral genotype was prevalent among others and caused the development of community-acquired adenoviral pneumonia. Phylogenetic analysis revealed a high degree of similarity with strains circulating in the United States and China, which does not rule out cross-border spread of the infection or evolutionary variability of the virus. Studies by foreign scientists show that outbreaks of adenovirus infection, caused primarily by adenoviruses B55 and B7, are frequently reported among military personnel, which causes significant eco-

nomical damage and reduces the army’s combat readiness [12–14, 16]. To prevent the disease and reduce hospitalizations and mortality among military personnel in the United States, an oral vaccine against adenoviruses B4 and B7 was developed and used in 1971; this vaccine helped curb outbreaks of adenovirus infection among recruits. However, production of this vaccine was discontinued in 1996 [9]. Several multivalent vaccines targeting adenovirus B55 are currently under development [23, 24].

### Conclusion

This study presents the results of genetic diversity analysis of adenoviruses detected in military personnel with acute respiratory viral infections (ARVI) during the 2023–2024 epidemiological season in the Sverdlovsk Region. Given the epidemiological significance of the B55 genotype, it is necessary to continue further molecular-genetic studies of adenoviruses circulating among military personnel in the Russian Federation. Given that adenovirus B55 leads to outbreaks of the disease among certain military contingents and causes severe disease progression, it is necessary to develop polyvalent adenovirus vaccines.

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