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Immunobiological properties of circulating *Bordetella pertussis* strains: Candidate strains for production of pertussis vaccines

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ABSTRACT

INTRODUCTION. One of the reasons for increased pertussis cases is the pathogen adapting to the existing collective immunity formed under conditions of vaccine prophylaxis. Monitoring immunobiological properties of *Bordetella pertussis* strains is necessary to track changes in the pathogen adaptive potential triggered by vaccination.

AIM. This study aimed to compare immunobiological properties of isolated circulating *Bordetella pertussis* strains and strains used to produce whole-cell pertussis vaccine.

MATERIALS AND METHODS. The study used nine isolates of modern circulating strains of *B. pertussis*. Experimental series of whole-cell pertussis vaccine was made using strains isolated from the patients with pertussis in 2016–2020. The series was evaluated by the following parameters: serological properties and antigenic structure (serotypes); hemagglutinating, hemolytic, and dermonecrotic effect; virulence; residual toxicity and protective properties. The study used outbred and inbred F1 mice (C57Bl/6J×CBA) and evaluated morphological and cultural properties of the bacterial culture. Experimental data were compared with the requirements for production strains set out in the local guidelines MUK 4.2.2317-08 (Selection, testing and storage of production strains of pertussis, parapertussis and bronchisepticosis bacteria).

RESULTS. Strains 16-16 and 33-18 were obtained from nine isolates of circulating *B. pertussis* strains meeting the requirements for production strains. The assessment results of protective potency for strains 25-16, 37-18, and 2-20 were analysed and showed the relevance of confirming this value due to the limited experimental material. Four *B. pertussis* strains, 31(2)-17, 28(1)-18, 25-16, and 2-20, did not show the required protective activity (<8 IU/mL).

CONCLUSIONS. The properties of isolates 16-16 and 33-18 of *B. pertussis* meet all the requirements for production strains. The test strains have a modern genotype and are prospectively applicable as candidates for replacing obsolete *B. pertussis* strains in production of pertussis vaccines.

Keywords: *Bordetella pertussis*; pertussis; serotype; incidence; virulence; whole-cell pertussis vaccine; vaccination; genotype; genome; circulating strains

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Иммунобиологические свойства циркулирующих штаммов *Bordetella pertussis*: кандидатные штаммы для изготовления коклюшных вакцин

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РЕЗЮМЕ

ВВЕДЕНИЕ. Одна из причин роста заболеваемости коклюшем заключается в адаптации патогена к имеющемуся коллективному иммунитету, сформированному в условиях вакцинопрофилактики заболевания. Мониторинг иммунобиологических свойств штаммов *Bordetella pertussis* необходим для прослеживания изменений адаптивного потенциала патогена в ответ на вакцинацию.
ЦЕЛЬ. Сопоставление иммунобиологических свойств выделенных изолятов циркулирующих штаммов *Bordetella pertussis* и производственных штаммов, используемых для изготовления цельноклеточной коклюшной вакцины.
МАТЕРИАЛЫ И МЕТОДЫ. В исследовании использованы 9 изолятов современных циркулирующих штаммов *B. pertussis,* выделенных от пациентов с коклюшем в 2016–2020 гг. Из штаммов изготовлены экспериментальные серии цельноклеточной коклюшной вакцины. Серии оценивали по следующим параметрам: серологические свойства и антигенная структура (серотипы); гемагглютинирующая, гемолитическая и дермонекротическая активности; вирулентность; остаточная токсичность и защитные свойства. В исследовании использовали аутбредных и инбредных мышей линии F1 (C57Bl/6J×CBA). Бактериальную культуру оценивали по морфологическим и культуральным свойствам. Экспериментальные данные сопоставляли с требованиями к производственным штаммам, изложенным в МУК 4.2.2317-08 (Отбор, проверка и хранение производственных штаммов коклюшных, паракоклюшных и бронхисептикозных бактерий).
РЕЗУЛЬТАТЫ. Из 9 изолятов циркулирующих штаммов *B. pertussis* выделены штаммы 16-16 и 33-18, которые соответствуют требованиям к производственным штаммам. Анализ результатов оценки защитной активности штаммов 25-16, 37-18 и 2-20 указал на целесообразность дополнительного подтверждения данного показателя из-за ограниченности опытного материала. Четыре штамма 31(2)-17, 28(1)-18, 25-16, 2-20 *B. pertussis* не проявили требуемой защитной активности (<8 МЕ/мл).
ВЫВОДЫ. Свойства изолятов 16-16 и 33-18 *B. pertussis* соответствуют всем требованиям к производственным штаммам. Исследованные штаммы имеют современный генотип и перспективны с точки зрения их практического использования в качестве кандидатов для замены устаревших производственных штаммов *B. pertussis* при изготовлении коклюшных вакцин.

Ключевые слова: *Bordetella pertussis*; коклюш; серотип; распространенность; вирулентность; цельноклеточная коклюшная вакцина; вакцинация; генотип; геном; циркулирующие штаммы

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INTRODUCTION

Pertussis is a highly contagious acute respiratory infection caused by *Bordetella pertussis* bacteria; historically, it was the leading cause of infant mortality worldwide [1, 2]. In the 1940–1950s, the use of whole-cell pertussis vaccine (WPV) in developed countries virtually eradicated pertussis. WPV reactogenicity, including sore injection site, irritability and fever, encouraged the development and implementation of acellular pertussis vaccine (APV) as a less reactogenic and more standardized drug. However, APVs did not live up to the expectations, and pertussis incidence increased following widespread APV immunization; first disease outbreaks were registered [3, 4]. There is a close correlation between the APV use and pertussis reoccurrence, which raises important questions about APV effectiveness and their ability to control morbidity [5–8].

There are several main reasons behind the increased pertussis incidence. Natural infection and vaccination induce different immunity types. Natural infection and WPV induce a T cell response with a shift to Th1/Th17, while APV shifts to Th2/Th17 [9]. This difference contributes to shorter immunity and reduced protection against infection when using APV compared to WPV. Thus, APV did not protect immunized baboon monkeys from colonization with *B. pertussis*, which allowed carriers to transmit the bacteria to uninfected individuals [10].

Changing host immunity and general epidemiological situation have an impact on the population of *B. pertussis* strains. Mooi et al. argued that the population of *B. pertussis* develops in response to collective immunity caused by APV, and that the pathogen adapting to the existing immunity level is one of the reasons of pertussis revival [6]. The adaptation of *B. pertussis* bacterial cells to post-vaccination immunity was noted earlier with the use of WPV, but with the transition to the APV use, adaptation significantly accelerated. Adaptation of *B. pertussis* bacterial cells to post-vaccinal immunity was previously registered for WPV; however, the transfer to APV significantly sped up the process. *B. pertussis* population adapts through mutations in the promoter regions of genes and genome regions encoding antigens, which are also part of the APV. Monitoring of circulating strains revealed *B. pertussis* strains with mutations in the genes encoding protective antigens, which contributes to immune evasion of the pathogen [7, 8, 11–16]. Alongside with the differing composition of nucleotides in the genes (alleles emerging for the same gene) of circulating strains and strains that are part of APV, immune evasion caused by the vaccine may be ascribed to a complete antigen loss.

This phenomenon was observed for pertactin, an outer membrane protein that promotes *B. pertussis* adhesion to the host epithelial cells [4, 17]. Given the differences in the structure of genes encoding protective antigens of circulating and vaccine strains, it is extremely important to detect a decreased effectiveness of pertussis vaccines in a timely manner. Thus, monitoring changes in the immunobiological properties of *B. pertussis* strains that cause further pathogen adaptation in response to vaccination [18] is a modern tool for monitoring the effectiveness of vaccination and immunoprophylaxis.

The aim of the study is to compare immunobiological properties of isolated circulating *Bordetella pertussis* strains and strains used to produce whole-cell pertussis vaccine.

MATERIALS AND METHODS

Materials

Strains. Isolates of currently circulating *Bordetella pertussis* strains 16-16, 31(2)-17, 28(1)-18, 25-16, 33-18, 37-18, 30-18, 1-20, and 2-20 obtained from children with pertussis were used

for the research. The strains were taken from the National Collection of Pathogenic Microorganisms, Scientific Centre for Expert Evaluation of Medicinal Products. The immunobiological properties of the strains were studied using vaccine batches of each strain. The vaccine batches were produced in accordance with pertussis vaccine manufacturing specification No. 136-69.

Serum samples. The serotype composition of strains and their ability to express typical agglutinogens (fimbriae) were determined using adsorbed type-specific dry pertussis sera to agglutinogens 1, 2, and 3 for the agglutination reaction (Microgen, Russia).

Cultural medium. *B. pertussis* strains were cultured in KUA (dry culture medium for pertussis strains) medium (Microgen, Russia). Sheep blood was added to the medium to a final concentration of 10%.

Reference standards. The following reference standards were used to evaluate the protective (immunogenic) and histamine-sensitizing activities: the pharmacopoeial reference standard for immunogenic activity of pertussis vaccine, FSO.3.2.00089[1]; histamine-sensitizing activity of pertussis vaccine, FSO.3.2.00087[2]; turbidity of 5 IU bacterial suspensions, FSO.3.1.00086[3].

Experimental animals. The tests were performed on outbred male and female mice weighing 15±1 g; on inbred male and female mice of F1 (C57Bl/6J×CBA) line weighing 11±1 g; and on 4-day-old outbred mice. The inbred mouse line was chosen based on its sensitivity to pertussis toxin. The animals were obtained from Andreevka branch of Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency and kept in an experimental room under standard conditions with *ad libitum* food and water. The temperature and relative humidity in the experimental room were 20–24 °C and 45–65%, respectively. Animal monitoring consisted of daily visits and recording of the animals' condition in the experimental record datasheets. When assessing the protective activity of *B. pertussis* strains, animal mortality was recorded. Body weight of mice was measured to assess residual toxicity. Dermonecrotic toxin was determined by measuring the area of hemorrhagic necrosis at the injection site of pertussis suspension. Animals withdrawn from the study were euthanized by introducing carbon dioxide into a special unit designed to ensure humane euthanasia. The study protocol using experimental animals was approved by the local Ethics committee of Scientific Centre for Expert Evaluation of Medicinal Products (Protocol No. 13 of August 11, 2025). All the studies were performed according to Directive 2010/63/EU of the European Parliament and the Council of the European Union on the protection of animals used for scientific purposes[4]; principles of the Council for International Organizations of Medical Sciences (CIOMS)[5]; European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes[6]; GOST 33216-2014[7]; Decision of Eurasian Economic Commission (EEC) No. 81 of November 3, 2016[8], EEC recommendations of November 14, 2023 No. 33[9]; General monograph OFS.1.7.2.0005.15[10] and Monograph FS.3.3.1.0010.15 of the State Pharmacopoeia of the Russian Federation[11].

Methods

The produced pertussis vaccines were evaluated based on the following parameters: serological properties and antigenic structure (serotypes); hemagglutinating, hemolytic, and dermonecrotic activity; virulence; residual toxicity

[1] FSO.3.2.00089 Immunogenic Activity of Whole-Cell Pertussis Vaccine.
[2] FSO.3.2.00087 Reference Standard of Histamine-Sensitizing Activity of Pertussis Vaccine.
[3] FSO.3.1.00086 Reference Standard of Turbidity for 5 IU Bacterial Suspensions.
[4] https://ruslasa.ru/wp-content/uploads/2017/06/Directive_201063_rus.pdf
[5] https://cioms.ch/
[6] European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. Strasbourg; 1986. https://norecopa.no/media/2iydns5h/ets-123-original.pdf
[7] GOST 33216-2014. Guidelines on Accommodation and Care for Laboratory Animals. Care and Husbandry Principles for Rodents and Rabbits.
[8] Decision of Eurasian Economic Commission No. 81 of 2016/11/03 On Approval of Good Laboratory Practice of the Eurasian Economic Union Regarding Drug Circulation.
[9] Recommendations of Eurasian Economic Panel No. 33 of 2023/11/14 On the Guidelines on Handling Laboratory (Experimental) Animals During Preclinical (Non-Clinical) Trials.
[10] General monograph OFS.1.7.2.0005.15 Immunogenicity of Pertussis Suspension and Whole-Cell Pertussis Component of Combined Vaccines. State Pharmacopoeia of the Russian Federation. Ed. XIV, vol. 2. Moscow; 2018.
[11] Monograph FS.3.3.1.0010.15 Diphtheria Pertussis Tetanus Adsorbed Vaccine (DPT Vaccine). State Pharmacopoeia of the Russian Federation. Ed. XIV. Vol. 4. Moscow; 2018.

(mouse body weight test); histamine-sensitizing activity (HSA), and protective properties. In addition, the morphological and cultural properties of *B. pertussis* bacterial culture were evaluated.

The isolates of circulating strains were examined according to the guidelines MUK 4.2.2317-08[12]. To assess the compliance of circulating strain isolates with the requirements for production strains, a whole-cell vaccine was prepared from each strain.

Statistical data were processed in Microsoft Office 2016. Protective activity data were processed by calculating geometric mean values. Specific safety was expressed as a relative value (%), calculated as the ratio of the body weight gain of vaccinated animals to the body weight gain of animals in the control group. Agglutinogen content corresponded to the final dilution of the pertussis vaccine or the titer at which the reaction with specific serum was completed by three crosses; HSA was expressed as an index calculated as the ratio HSD_{50FSO} to $HSD_{50vaccine}$. HSD_{50FSO} denotes the dose of histamine-sensitizing activity for pertussis vaccine by General monograph FSO.3.2.00087, corresponding to the 50% death of immunized animals upon histamine administration. $HSD_{50vaccine}$ denotes the vaccine dose corresponding to the death of 50% of immunized animals upon histamine administration.

RESULTS AND DISCUSSION

The genotypic characteristics of the studied production and circulating strains of *B. pertussis* was first introduced by O.Yu. Borisova et al. [19]. *Table 1* shows the results of an additional analysis of previously obtained data, demonstrating that the genotypes of production strains differ significantly in the range of allelic variants for protective antigens from the genotypes of circulating strains. Thus, some strains are common for *ptxA*1, *ptxB2*, and *fim3*-1, while the studied strains differ in other alleles of the genes encoding the protective antigens ptxC, ptxP, prn, and fim2 *(Table 1)*.

In accordance with the guidelines MUK 4.2.2317-08[13], the following parameters of *B. pertussis* circulating strains were studied: Morphology, Culture properties, Serological

Table 1. Genotypic profile of production and *Bordetella pertussis* circulating strains
Таблица 1. Генотипическая характеристика производственных и циркулирующих штаммов *Bordetella pertussis*

***B. pertussis* strains** *Штаммы B. pertussis*	**Allele gene variants / *Аллельные варианты генов***						
	***ptxA*[a]**	***ptxB*[b]**	***ptxC*[c]**	***ptxP*[d]**	***Prn*[e]**	***fim2*[f]**	***fim3*[g]**
Production strains *Производственные*	*ptxA1* *ptxA2* *ptxA4*	*ptxB1* *ptxB2*	*ptxC1*	*ptxP1* *ptxP2*	*prn1*	*fim2-1*	*fim3-1*
Circulating strains *Циркулирующие*	*ptxA1*	*ptxB2*	*ptxC2*	*ptxP3*	*prn2* *prn9*	*fim2-2*	*fim3-1* *fim3-2*

The table was prepared by the authors using data of O.Yu. Borisova et al. [19] with additions / Таблица составлена авторами по данным О.Ю. Борисовой с соавт. [19] с дополнениями

Note
[a] *ptxA,* pertussis toxin gene allele encoding the S1 subunit;
[b] *ptxB,* pertussis toxin gene allele encoding the S2 subunit;
[c] *ptxC,* pertussis toxin gene allele encoding the S3 subunit;
[d] *ptxP,* pertussis toxin promoter gene allele;
[e] *prn,* pertactin gene allele;
[f] *fim2,* fimbria 2 gene allele;
[g] *fim3,* fimbria 3 gene allele.

Примечание
[a] *ptxA* – аллель гена коклюшного токсина, кодирующий S1-субъединицу;
[b] *ptxB* – аллель гена коклюшного токсина, кодирующий S2-субъединицу;
[c] *ptxC* – аллель гена коклюшного токсина, кодирующий S3-субъединицу;
[d] *ptxP* – аллель гена промотора коклюшного токсина;
[e] *prn* – аллель гена пертактина,
[f] *fim2* –аллель гена фимбриального белка фимбрии 2;
[g] *fim3* – аллель гена фимбриального белка фимбрии 3.

[12] MUK 4.2.2317-08 Selection, testing and storage of production strains of pertussis, parapertussis and bronchisepticosis bacteria. Moscow; 2009.
[13] Ibid.

properties, Antigenic structure (serotypes), Hemagglutinating and hemolytic activity, Dermonecrotic activity, Virulence, Residual toxicity, and Potency.

Morphological and culture properties of the isolated strains met the requirements for production strains. Such properties are typical for the smooth S-form (phase I) of *B. pertussis* bacteria. Other immunobiological properties of the circulating strains are presented in *Table 2*.

Serotype composition. The evaluated antigenic structure showed that the vast majority of *B. pertussis* strains had a 1.0.3 serotype composition; all isolated strains actively expressed their characteristic fimbriae. The cultures were agglutinated with the appropriate adsorbed type-specific sera for agglutinogens 1, 2, and 3 at a serum dilution of ⩾1:2,560. In accordance with regulatory requirements, the serum dilution should be at least 1:1,280. The production strains had a composition of serotypes 1.2.3, 1.2.0, and 1.0.3. Circulating strains included no bacteria expressing fimbriae 2 and 3 simultaneously, that is, serotype 1.2.3 was not detected. Thus, six of the eight circulating strains had serotype composition 1.0.3, and the remaining two strains – serotype composition 1.2.0.

Table 2. Immunobiological properties of circulating strain isolates
Таблица 2. Иммунобиологические свойства изолятов циркулирующих штаммов

Strain, No. *Штамм, №*	**Agglutinogens (fimbriae), titer** *Агглютиногены (фимбрии), титр*	**Hemagglutinating activity, billion** *Гемагглютинирующая активность, млрд*	**Virulence, million microbial cells** *Вирулентность, млн мкр. клеток*	**Specific safety, %** *Специфическая безопасность, %*	**Histamine-sensitizing activity, index** *Гистаминсенсибилизирующая активность, индекс*	**Potency, IU/mL** *Защитная активность, МЕ/мл*
16-16	1f. 1:5120–1:10240 2f. not detected / *не выявлен* 3f. 1:5120–1:10240	10–20 (3+)	1.903	66.7–96.0	0.49	10.5
31(2)-17	1f. 1:2560–1:5120 2f. not detected / *не выявлен* 3f. 1:5120–1:10240	20 (3+)	0.617	75.7–96.0	0.28	4.7
28(1)-18	1f. 1:2560–1:5120 2f. not detected / *не выявлен* 3f. 1:5120–1:10240	20 (3+)	3.623	67.8–76.0	0.63	5.1
25-16	1f. 1:2560–1:5120 2f. not detected / *не выявлен* 3f.1:5120	5 (3+)	2.626	67.7–90.7	0.73	12.5
33-18	1f. 1:2560–1:5120 2f. 1:2560–1:5120 3f. not detected / *не выявлен*	5 (3+)	1.274	69.7–99.7	0.52	10.4
37-18	1f. 1:5120 2f. 1:2560 3f. not detected / *не выявлен*	10 (3+)	0.851	67.3–99.6	0.37	8.7
30-18	1f. н/о / *n/d* 2f. н/о / *n/d* 3f. not detected / *не выявлен*	5 (3+)	1.940	62.4–96.6	0.71	6.7
1-20	1f. 1:2560 2f. not detected / *не выявлен* 3f. 1:2560	0.313 (3+)	n/d *н/о*	69.1–95.1	n/d *н/о*	5.3
2-20	1f. 1:2560 2f. not detected / *не выявлен* 3f. 1:5120	0.313 (3+)	2.626	62.5–99.6	n/d *н/о*	7.5

The table was prepared by the authors using their own data / Таблица составлена авторами по собственным данным

Note. 1f , factor 1; 2f, factor 2; 3f, factor 3; n/d, not determined; IU, international units.
Примечание. 1f — фактор 1; 2f — фактор 2; 3f — фактор 3; н/о — не определяли; мкр. клетки — микробные клетки; МЕ — международные единицы.

Hemagglutinating activity. All *B. pertussis* strains showed hemagglutinating activity and agglutinated sheep erythrocytes by three crosses (3+). According to regulatory requirements, a pertussis suspension with 10 IU opacity should agglutinate sheep erythrocytes by 2+. Hemagglutinating activity allows distinguishing the following active strains: 16-16, 31(2)-17, 28(1)-18, 37-18 agglutinated as 3+ at a concentration of 10–20 billion/mL; strains 25-16, 33-18, and 30-18 – at a concentration of 5 billion/mL; strains 1-20 and 2-20 showed the greatest activity and agglutinated by 3+ at the dilution of 0.313 billion/mL.

Hemolytic activity. All strains showed hemolytic activity. Single colonies of *B. pertussis* bacterial cells in a thin layer of Bordet – Gengou medium were surrounded by a hemolysis zone.

Dermonecrotic activity was confirmed by subcutaneous administration of the culture to 4-day-old outbred mice. Hemorrhagic necrosis formed at the injection site of a live culture at a concentration of 20 billion/mL, that corresponded to the regulatory requirements for *B. pertussis* production strains.

Virulence. The culture of *B. pertussis* should be virulent for mice. In case of intracerebral injection, LD_{50} value should not exceed 25 million microbial cells. Circulating strains showed high virulence, since LD_{50} values ranged from 3.623 to 0.851 million microbial cells[14].

Residual toxicity of the strains is caused by the presence of residual amount incompletely neutralized pertussis toxin in the vaccine and the presence of a lipooligosaccharide. The parameter was determined using a WHO-recommended mouse weight test and by assessing histamine-sensitizing activity (HSA). Residual toxicity was assessed throughout the entire shelf life of the vaccine (1 year). At the beginning of the shelf life, residual toxicity that reflects the residual toxicity of the drug, was almost at the minimum acceptable level. According to guidelines MUK 4.2.2317-08[15], the parameter should be ⩾60%. However, by the end of the shelf life, the values for almost all circulating strains increased to ⩾90%, suggestive of an effective detoxification of pertussis toxin and prolonged effect of formaldehyde added to the vaccine to neutralize *B. pertussis* toxins. This observation indicates that in order to obtain a less reactive DPT vaccine for mixing with diphtheria and tetanus components, a pertussis suspension should be used that was previously stored for as long as possible (preferably during the entire shelf life of one year) in conditions of pertussis toxins detoxification [20]. The addition of formaldehyde and the mode of its action must be taken into account in order to obtain a safer pertussis vaccine preparation [20].

Potency. Potency evaluation of pertussis vaccines obtained from isolates of circulating strains shows that 16-16 and 33-18 *B. pertussis* strains have a significantly strong potency *(Table 2)*. This meets the regulatory requirement that the potency should be ⩾8 IU/mL. Potency assessment results for 25-16, 37-18, and 2-20 *B. pertussis* strains need to be confirmed due to the limited experimental material. The remaining four strains have a weak potency (<8 IU/mL).

Thus, the compliance test for immunobiological properties of *B. pertussis* circulating strains isolates with regulatory requirements for production strains shows that all nine studied strains have a set of properties typical for the smooth (S-form) bacteria. All isolated strains actively express agglutinogens (fimbriae) and show hemagglutinating, hemolytic, histamine-sensitizing, and dermonecrotic action, pronounced virulence and low residual toxicity (at the end of the shelf life). Two of the nine studied strains demonstrated the required potency.

Significant differences between production and circulating strains were revealed when studying their genotypic diversity. The earlier data obtained by the authors [19] are consistent with foreign references [7, 21] and indicate that the alleles of pertactin *prn2*, pertussis toxin *ptxA1*, and pertussis toxin promoter *ptxP3* genes are dominant. Circulating *B. pertussis* bacteria carrying these alleles may have advantages in a population of individuals vaccinated with APV containing obsolete vaccine strains. This factor may affect vaccines by reducing their effectiveness.

Currently, *B. pertussis* bacteria carrying the *ptxP3* allele are considered the global cause of epidemics. Strains with the *ptxP3* allele that are now ubiquitous were first discovered in the late 1980s. In a number of countries, the prevalence of such strains is >90% leading

[14] MUK 4.2.2317-08 Selection, testing and storage of production strains of pertussis, parapertussis and bronchisepticosis bacteria. Moscow; 2009.
[15] Ibid.

to replacement of *B. pertussis* population carrying the *ptxP1* allele [22–24]. These strains show a characteristic resistance to macrolides [12]. In addition, strains with the *ptxP3* allele produce 1.6 times more pertussis toxin than strains with the *ptxP1* allele [25].

A more intensive production of pertussis toxin by *B. pertussis* strains with the *ptxP3* allele compared to *ptxP1* explains their rapid global distribution. Pertussis toxin plays a key role in suppressing the innate and acquired immunity [26]. On the one hand, increased production of pertussis toxin enhances an effective immune response by increasing pathogen transmission and, consequently, the pathogen adaptation. On the other hand, increased toxin production may be beneficial for the pathogen, since the body is forced to produce higher levels of specific neutralizing antibodies. Pertussis toxin causes pathological leukocytosis associated with increased infant mortality due to the developing pulmonary hypertension [27]. Thus, spreading *B. pertussis* strains with the *ptxP3* allele may increase pertussis incidence and mortality, with an evidence of high virulence of these strains [25, 28].

Thus, the previously expressed opinion that an increased pertussis incidence is mainly due to a weakened immune system is not complete. Since vaccination has been introduced, significant changes were noted in the *B. pertussis* populations, presuming pathogen adaptation in pertussis recurrence and maintenance. The adaptation includes antigenic divergence with vaccine strains and increased production of pertussis toxin. Antigenic divergence affects the formation of memory T cells and the ability of antibodies to effectively recognize the antigen.

Higher levels of pertussis toxin may enhance the suppression of the body immune response. Apparently, this adaptation of *B. pertussis* led to a shorter period of effective action of pertussis vaccines and accelerated immunocompromise [6]. Thus, vaccines containing obsolete vaccine strains cannot effectively protect the population from modern circulating *B. pertussis* strains, resulting in an increased pertussis incidence and epidemic outbreaks. For example, in the Russian Federation, pertussis incidence rate was 36.2 per 100,000 inhabitants in 2023, which is 16.4 times higher than in 2022[16] [29].

Antigenic polymorphism analysis of clinical isolates indicates the feasibility of regular replacement of production strains with strains that currently predominate in the population [6, 30]. The Strategy for the development of immunoprophylaxis of infectious diseases for the period up to 2035[17] emphasizes the need to create, maintain and replenish the bank of microbial production strains in order to supply manufacturers of immunobiological preparations with samples of production strains. Isolated *B. pertussis* strains 16-16 and 33-18 have a modern genome and comply with regulatory requirements for industrial pertussis strains (MUK 4.2.2317-08[18])

These strains may be considered as candidates for introduction into a Russian WPV in order to replace the obsolete production strains and may be used in the APV production. Monitoring of circulating *B. pertussis* strains is important to identify a range of genetic changes that affect the pathogen adaptation under vaccination conditions [18]. The permanent evolution of *B. pertussis* genome warrants an integrated approach that includes analysis of vaccine characteristics and data on circulating strains monitoring as well as interactions between strains to solve the problem of the increased pertussis incidence.

CONCLUSIONS

1. Immunobiological properties of circulating *B. pertussis* isolates and production strains used to manufacture whole-cell pertussis vaccines isolated between 2016 and 2020 have been analyzed.
2. Analyzed immunobiological properties of circulating *B. pertussis* strains show that isolates 16-16 and 33-18 meet all the requirements for production strains outlined in the local guidelines 4.2.2317-08 Selection, testing and

[16] Rospotrebnadzor Information Letter No. 02/1860-2024-27 of 2024/02/06 On Arranging External Quality Control of Diagnostic Studies for Diphtheria and Pertussis over 2024 in the Far Eastern and Ural Federal District.
Analytical Data of Reference Centre for Pertussis and Diphtheria Monitoring in G.N. Gabrichevsky Research Institute for Epidemiology and Microbiology, Form 2, Data on Infectious and Parasitic Diseases.
On Public Sanitation and Disease Control in the Russian Federation, 2021: State report. Moscow; 2022.

[17] Decree of the Government of the Russian Federation No. 2390-r as of 2020/09/18 On Approving the Strategy for the Development of Immunoprophylaxis of Infectious Diseases for the Period up to 2035.

[18] MUK 4.2.2317-08 Selection, testing and storage of production strains of pertussis, parapertussis and bronchisepticosis bacteria. Moscow; 2009.

storage of production strains of pertussis, parapertussis and bronchisepticosis bacteria.
3. From the practical point of view, *B. pertussis* strains 16-16 and 33-18 possess a modern genotype and are promising candidates for replacement of obsolete production strains while manufacturing pertussis vaccines.
4. Regular monitoring of the genotypes and immunobiological properties of circulating *B. pertussis* strains appears relevant in order to promptly replace obsolete strains in the modern prophylactic vaccines, since *B. pertussis* constantly adapts to the existing level of immunity in response to collective immunity.

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