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Comparative genomic analysis of clinical isolates of Klebsiella pneumoniae isolated from newborns with different outcomes of the infectious process in the neonatal period

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Abstract

Introduction. Some progress has been made in the study of the molecular mechanisms of antibiotic resistance, namely, genes and their variants have been identified that ensure the inactivation of beta-lactam antibiotics. Nevertheless, there is still a necessity for further studies of genetic diversity of nosocomial strains, prevalence of genetic determinants of resistance to other groups of antibiotics, virulence factors and realization of pathogenic potential by opportunistic microorganisms.

Aim of the study was to compare the genetic profile of clinical isolates of *Klebsiella pneumoniae* isolated from newborns with different outcomes of the infectious process in the neonatal period.

Materials and methods. Using whole-genome sequencing and bioinformatic analysis to search for determinants of resistance and virulence, 3 strains of *K. pneumoniae* were studied, 2 of which were isolated from the blood of a generalized form of infection, 1 from the feces of a newborn child.

Results. *K. pneumoniae* strains belonged to sequence types (ST) ST23, ST14 and ST3559, and differed in genetic determinants of antibiotic resistance and virulence factors. At the same time, they all had the genetic determinants *fimH*, *mrkA* and *iutA*, which are associated with an increased ability to attach to substrates and transport aerobactin. Strain 222 of ST3559, which has the largest number of antibiotic resistance genes, contained the smallest number of virulence factor genes, and vice versa, strain 144 of ST23, in which the smallest number of antibacterial drug resistance genes was detected, contained the most virulence factor genes.

Conclusions. Identification of *K. pneumoniae* strains that differ in the genetic profile of antibiotic resistance and virulence in neonatal hospital patients indicates a complex interaction between bacteria and the macroorganism, in which isolates with low pathogenic potential can cause serious infectious complications, and vice versa, when a highly virulent strain does not realize its pathogenic potential, as demonstrated in case of *K. pneumoniae* strains ST14, ST3559 and ST23, respectively. This highlights the difficulty of effectively predicting and managing infection risks in hospital operations.

Keywords: *Klebsiella pneumoniae, next-generation sequencing, bioinformatics analysis, virulence genes, antibiotic resistance genes*

Ethics approval. The study was conducted with the informed consent the legal representatives of patients. The research protocol was approved by the Ethics Committee of the Ural Scientific Research Institute of Maternity and Child Care (protocol No. 15, December 6, 2022).

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Оригинальное исследование
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Сравнительный геномный анализ клинических изолятов *Klebsiella pneumoniae*, выделенных от новорождённых детей с различными исходами инфекционного процесса в неонатальном периоде

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Аннотация

Введение. Существует необходимость исследования генетического разнообразия внутрибольничных штаммов, распространённости детерминант устойчивости к антибиотикам, факторов вирулентности и реализации патогенного потенциала оппортунистическими микроорганизмами.

Цель работы — сравнить генетический профиль антибиотикорезистентности и вирулентности клинических изолятов *Klebsiella pneumoniae*, выделенных от новорождённых детей с различными исходами инфекционного процесса в неонатальном периоде.

Материалы и методы. С помощью полногеномного секвенирования и биоинформационного анализа для поиска детерминант резистентности и вирулентности исследованы 3 штамма *K. pneumoniae*, 2 из которых выделены из крови при генерализованной инфекции, 1 — из фекалий новорождённого ребёнка.

Результаты. *K. pneumoniae* ST23, ST14, ST3559 отличались генетическими детерминантами антибиотикорезистентности и факторов вирулентности. Вместе с тем все они имели гены *fimH*, *mrkA* и *iutA*, ассоциированные с повышенной способностью к адгезии к субстратам и транспортом аэробактина. Штамм ST3559, обладающий наибольшим количеством генов антибиотикорезистентности (9), содержал 8 генов факторов вирулентности; в штамме ST23, в котором детектировано наименьшее количество генов устойчивости к антибактериальным препаратам (3), обнаружено больше всего генов факторов вирулентности (21).

Заключение. Выявление штаммов *K. pneumoniae*, различающихся по генетическому профилю антибиотикорезистентности и вирулентности, у пациентов неонатальных стационаров указывает на сложное взаимодействие между бактериями и организмом новорождённого ребёнка, при котором изоляты с низким патогенным потенциалом могут вызывать серьёзные инфекционные осложнения, и наоборот, когда высоковирулентный штамм не реализует свой патогенный потенциал, как в случаях с *K. pneumoniae* ST14, ST3559 и ST23. Это подчёркивает сложность эффективного прогнозирования и управления инфекционными рисками в деятельности стационаров.

Ключевые слова: *Klebsiella pneumoniae, полногеномное секвенирование, биоинформационный анализ, гены вирулентности, гены антибиотикорезистентности*

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Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

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Introduction

Klebsiella pneumoniae is a typical representative of *Enterobacteriaceae*, which can be detected during asymptomatic colonization of mucous membranes of non-sterile biotopes of the human body [1]. At the same time, *K. pneumoniae* is included in the top five etiologic agents associated with fatal infectious processes worldwide, regardless of the antibiotic sensitivity of the isolate [2]. According to the results of a multicenter epidemiological study in Russia, *K. pneumoniae* is the most common bacterial pathogen of nosocomial infections of the respiratory (35.81%) and urinary (31.94%) systems, cardiovascular (26.40%), central nervous system infections (CNS; 27.78%), and is the second most common pathogen of nosocomial infections of skin and soft tissues (19.10%), abdominal cavity (26.26%), and bone and joint infections (15.93%) [3].

Among *Enterobacteriaceae*, which are etiologic agents of complications of infectious genesis in newborns in the intensive care unit, *K. pneumoniae* is registered in 48% of cases [4]. In children treated in the hospital of Kemerovo region, it was most often detected in etiologically significant titers in fecal samples (826.41 per 1000 patients) and pharyngeal secretions (33.96 per 1000 patients) [5]. In a pediatric hospital located in Nizhny Novgorod, the epidemiological situation is associated with *K. pneumoniae* sequence type (ST) ST3181, originally isolated in Australia and first described in Russia [6].

K. pneumoniae is the 3rd most frequent etiologic agent of bloodstream infections after *Staphylococcus aureus* and coagulase-negative staphylococci in pediatric departments of the Republic of Belarus, where it is registered in 14.6% of cases [7].

In other countries, where the mortality rate from generalized bloodstream infections is registered at 18–68%, *K. pneumoniae* is also one of the significant pathogens found in newborns hospitalized in intensive care units [8].

When analyzing genetic variants, it was found that in one of the pediatric hospitals in Moscow, *K. pneumoniae* strains belonged to 4 sublines: SL307, SL395, SL29 and SL1198, which indicates the heterogeneity of the strain population and the possible presence of several variants of one bacterial species at once in the conditions of a pediatric ward [9].

The detection of resistance genes to all categories of antibiotics recommended for therapy of *Enterobacteriaceae* in the genomes of 6 panresistant strains once again confirms the urgency of the problem of finding drugs for effective antibiotic therapy. In Morocco, during active surveillance of rectal carriage in newborns, 91 (31.05%) of 293 collected *K. pneumoniae* isolates were found to produce carbapenemase. Among carbapenem-resistant *K. pneumoniae*, 37 (40.65%) contained the bla_{OXA-48} gene; bla_{NDM}, bla_{VIM} and bla_{KPC} genes were detected in 30.76, 9.89 and 2.19% of the isolates, respectively [10]. Globally prevalent multidrug-resistant sequence types include ST14/15, ST17/20, ST43, ST147, ST258 and ST395 [11], with the latter, frequently encountered in pediatric inpatients, being associated with colistin resistance [12]. The detection of convergent types is noteworthy. For example, hospital outbreaks in 2 hospitals in St. Petersburg were caused by carbapenem-resistant hypervirulent strains [13]. In Moscow, ST395 was detected, combining features of both antibiotic-resistant and virulent microorganisms capable of dissemination in the human body [9].

Based on the above mentioned information, *K. pneumoniae* is a relevant opportunistic microorganism associated with the occurrence of both hospital-acquired and out-of-hospital infections. The reason for this is the high rate of transmission of genetic determinants of virulence and antibiotic resistance through mobile genetic elements, the formation of pathogenic and/or antibiotic-resistant epidemically significant clonal lineages and their spread among patients worldwide [9].

Perinatal centers are not an exception and logically fit into the system of medical care at the inpatient stage, within their walls being a contingent with limited therapeutic capabilities and a high risk of infectious and inflammatory processes caused by opportunistic microorganisms. This is due to the morphofunctional immaturity of various organs and their systems in children born low birth weight and/or from early and ultra-early premature births [14].

The study of molecular mechanisms of antibiotic resistance has made progress: genes and their variants that ensure inactivation of antibacterial drugs have been identified, and association with certain clonal groups has been established. Nevertheless, there is still a necessity for further studies of genetic diversity of nosocomial strains, prevalence of genetic determinants of antibiotic resistance, virulence factors and realization of pathogenic potential by opportunistic microorganisms.

The aim of the study was to compare the genetic profile of antibiotic resistance and virulence of clinical isolates of *K. pneumoniae* isolated from newborn infants with different outcomes of the infectious process in the neonatal period.

Materials and methods

The study was approved by the local ethical committee of the Research Institute of Maternal and Infant Health Protection (protocol No. 15 of 06.12.2022).

Three strains of *K. pneumoniae*, 2 of which were isolated from blood during late hospital neonatal sepsis, and the other one from feces of a child during local microbiological monitoring[1]. It should be noted that the bloodstream infection was fatal in one case, but a full recovery was observed in the other case. The strains were isolated on 05.04.2023, 11.10.2023, 26.02.2024 and stored in the collection of the microbiology laboratory. Nucleotide sequences were deposited in GenBank international database of genetic information (BioProject: PRJNA1144786, GenBank numbers: JBGKAX0000000000000, JBGKAY000000000, JBHILO0000000000000).

Blood was collected in a volume of up to 4 ml from an intact vein into a pediatric vial directly at the patient's bedside and subsequently cultured in a BacT/ALERT analyzer (bioMerieux).

Positive hemocultures and feces were sown on nutrient media: Endo, differential-diagnostic lactose-containing nutrient medium (State Research Center for Applied Microbiology and Biotechnology) and blood-serum agar (base — Conda).

Species identification of bacteria and determination of sensitivity to antibacterial drugs (ampicillin, amoxicillin + clavulanic acid, cefotaxime, ceftazidime, cefepime, ertapenem, meropenem, amikacin, gentamicin, ciprofloxacin, tigecycline, fosfomycin, nitrofurantoin, trimethoprim sulfamethoxazole, colistin) were performed on a VITEK 2 compact automatic bacteriological analyzer (Bio Mérieux) at the CCU of the Innovative Scientific Laboratory Center for Perinatal and Reproductive Medicine, using VITEK 2 GN (identification) and AST-N360 (antibiotic sensitivity determination) test cards.

To assess the biofilm-forming ability of bacteria, we used the method described previously [15].

Total DNA was isolated from 24-hour culture using D-Cells-10 kits (Biolabmix LLC). Sequencing of strains 222 and 56 was performed on the MiSeq platform (Illumina), and strain 144 was sequenced on the SURFSeq 5000 (GeneMind). The quality of reads was assessed using the FastQC program tool [16]. *De novo* genome assembly was performed using midsystem [17]. Multilocus sequencing was performed according to the method proposed by the Pasteur Institute [18]. Analysis of DNA nucleotide sequences of 7 housekeeping genes: *rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB*, *tonB* and other loci of the genome of *K. pneumoniae* were analyzed using the BIGSdb-Pasteur database of the Pasteur Institute[2].

Genetic determinants of antibiotic resistance and virulence were searched using online services: VirulenceFinder[3] and ResFinder[4]. Typing of capsule loci (C-loci) was performed using the Kaptive site[5] [19].

For comparative analysis of the sequences we obtained, we used the data of GenBank NCBI.

Hyperproduction of mucus was determined using the methodology described in [20].

Results

Brief characterization of patients

K. pneumoniae strain No. 222 was isolated from a positive hemoculture of patient P. on the 49th day of life. Its detection from a blood sample with clinically expressed generalized infection was preceded by a 10-day colonization of the intestine with *K. pneumoniae*, established by local microbiologic monitoring.

K. pneumoniae No. 56, detected in patient M., was initially isolated from a fecal sample on the 35th day of life during local microbiological monitoring. From somatic pathology it should be noted the presence of CNS hypoxia at birth and intrauterine malformations of the CNS and cardiovascular system, which aggravated the child's condition in the neonatal period. At the age of the child 43 days *K. pneumoniae* in monoculture was isolated from the contents of his tracheobronchial tree and from positive hemoculture with negative dynamics of the clinical condition of the newborn, which confirms the translocation of the strain through the intestinal wall and its dissemination throughout the body. On the 44th day, death has occurred, and during bacteriological examination of sectional material (blood from the heart cavity, intestinal tissue, lungs, liver) *K. pneumoniae* in monoculture was isolated from all samples of the listed biological material without accompanying microorganisms.

K. pneumoniae strain No. 144 was isolated during local microbiological monitoring of the department of newborn premature babies from feces of patient Sh. (date of birth 21.02.2024) on the 6th day of his life (gestational age: 36.5 weeks, body weight 2650 g, Apgar score 5/7 during the 1st and 7th minutes of life). During the whole neonatal period of the child in the hospital the results of laboratory studies were without signs of inflammatory process. After discharge in a satisfactory condition home on the 15th day of life, the family did not seek medical help during 3 months, which indicates the absence of invasive infectious processes. This condition was determined both by the ability of immunological reactions to timely recognize antigen and ensure the maintenance of antigenic homeostasis, and by the morphological characteristics and phenotypic properties of the bacterial agent.

Phenotypic characterization of strains

Optical density values obtained during the study of biofilm-forming ability of strains, information on

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[1] Order of the Federal State Budgetary Institution "Ural Research Institute for Maternal and Child Health" of the Ministry of Health of Russia No. 263-p dated 26.06.2016 on the procedure for microbiological monitoring.

[2] URL: https://bigsdb.pasteur.fr/klebsiella

[3] URL: https://cge.cbs.dtu.dk/services/VirulenceFinder

[4] URL: https://cge.cbs.dtu.dk/services/ResFinder

[5] URL: https://kaptive-web.erc.monash.edu

Table 1. Metadata and phenotypic characterization of *K. pneumoniae* strains

Parameter	Strain number		
	222	56	144
Date of discovery	05.04.2023	11.10.2023	26.02.2024
Patient	P.	.	Sh.
Colonization of the intestine with *K. pneumoniae* preceding the infectious process	Yes	Yes	Yes
Nosological form	Generalized infection	Generalized infection	Carriage (colonization of the intestinal biotope)
Outcome	Recovery	Death	Carriage (colonization of the intestinal biotope)
Biofilm formation, optical density, nm	0.235	0.045	0.555
Hyperproduction of mucus	No	No	Yes

mucus hyperproduction, forms and outcomes of nosologies, and other metadata are summarized in **Table 1**.

As shown in the data presented in Table 1, the studied strains were isolated from samples of biological material with an interval of several months. Colonization of the intestinal biotope was observed in all patients, and in two of them the generalization of the infectious process was registered. It ended lethally for one of the patients. Hyperproduction of mucus, noted in 1 of 3 strains, is associated with increased ability of biofilm formation.

One of the significant properties of bacterial strains, including for clinicians, is antibiotic sensitivity.

The minimum inhibitory concentrations of antibacterial drugs of strains No. 222 and 56 are presented in **Table 2**. Strain No. 144 was sensitive to all tested antibiotics except ampicillin.

Strains isolated from positive hemoculture developed resistance to protected amoxicillin (Table 2). Strain No. 222 exhibited multidrug resistance and produced extended-spectrum beta-lactamases (ESBLs). Having developed resistance to one of the aminoglycosides (gentamicin), ciprofloxacin and chloramphenicol, it remained sensitive to colistin, fosfomycin, amikacin and antibiotics from the carbapenem group (ertapenem and meropenem).

Table 2. *K. pneumoniae* strains No.56 and 222 sensitivity to antimicrobial drugs

Antibiotic	Strain number			
	222		56	
	minimum inhibitory concentration, mg/L	sensitivity	minimum inhibitory concentration, mg/L	sensitivity
Ampicillin	≥ 32	–	≥ 32	–
Amoxicillin clavulanate	≥ 32	–	≥ 32	–
Cefotaxime	≥ 64	–	≤ 0.25	+
Ceftazidime	32	–	16	–
Cefepime	≥ 32	–	≤ 0.12	+
Ertapenem	≤ 0.12	+	≤ 0.12	+
Meropenem	≤ 0.25	+	≤ 0.25	+
Amikacin	4	+	≤2	+
Gentamicin	≥ 16	–	≤ 1	+
Ciprofloxacin	1	–	≤ 0.25	+
Fosfomycin	≤ 16	+	≤ 16	+
Trimethoprim	≥ 320	–	≤ 0.20	+
Colistin	≤ 0.5	+	≤ 0.5	+

Table 3. Comparative genetic characteristics of strains isolated from newborns with different outcomes of the infectious process

Parameter	Strain number		
	222	56	144
Genome size, bp	5 414 099	5 544 559	5 468 329
GC composition, %	57.3	57.1	57.4
ST	3559	14	23
KL type	KL27	KL2	KL1
O locus	O4	O1/O2v1	O3/O3a
O type	O4	O1	O3/O3a
Number of genes	5299	5311	5176
Number of contigs	101	89	79
Antibiotic resistance genes	*aac (6')-Ib-cr* $bla_{CTX\text{-}M\text{-}15}$ $bla_{SHV\text{-}11}$ $bla_{TEM\text{-}1B}$ *fosA6* *oqxA.B* *blaOXA-1* *catB3* *dfrA1*	$bla_{SHV\text{-}1}$ *fosA* *oqxA.B* *tet(D)* *catA1*	$bla_{SHV\text{-}190}$ *fosA6* *oqxA.B*
Virulence genes	*fimH,* *iutA,* *traT* *fyuA* *irp1, 2* *kfuA, B* *mrk A, B, C, D, F, H, I, J* *ybt A, E, P, Q, S, T, U, X*	*fimH,* *iutA* *traT* *kfuA, B, C* *mrkA, B, C, D, F, H, I, J*	*fimH,* *iutA* *mchF* *allA, B, C, D, R, S* *arcC* *clbA, B, C, D, E, F, G, H, I, L, M, N, O, P, Q, R* *fdrA* *fyuA* *gcl* *glxK, R* *hyi* *iroB, C, D, N,* *irp1, 2* *iucA, B, C, D, A* *kfuA, B, C* *mceA, B, C, D, E, G, H, I, J* *mrkA, B, C, D, F, H, I, J* *rmpA, A2, ybbW, Y, A, E, P, Q, S, T, U, X* *ylbE, ybtA, E, P, Q, S, T, U, X F*
Virulence score	1	0	5
Antimicrobial resistance score	1	0	0
Incompatibility groups of plasmids	IncFIB(K), IncFII(K)	IncFIB(K)	IncHI1B, IncFIB(K)

The genetic determinants of antibiotic resistance that cause phenotypic resistance to antibiotics, along with genes for virulence factors, external and internal structures of bacterial cells, provide more information for in-depth analysis of microbial data.

Genetic characterization of strains

As can be seen from the data presented in **Table 3**, the studied strains belong to three STs: ST3559, ST14, ST23.

Strain No. 222 of ST3559 belongs to clonal group CG429, being a variant of sublineage ST429 widespread through all continents [21]. This strain scores 1 in virulence due to the presence of *ybt* gene and 1 in antibacterial resistance due to the ESBL gene.

Strain No. 56 of ST14 has been reported as an etiologic agent of neonatal sepsis in central Italy [22], Turkey [23], Vietnam [14], India [24] and Tanzania [25], confirming its widespread prevalence in pediatric units.

Strain No. 144 of ST23 belongs to the hypervirulent clonal group CG23, sublineage SL23. It was characterized by 5 points out of 5 in virulence assessment due to the presence of genes encoding colibactin syn-

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thesis (*clb1*), aerobactin synthesis (*iuc1*) and yersiniabactin synthesis (*ybt*). It is the most virulent strain among those that were compared in this study.

Discussion

Strains No. 56 and 222 phenotypically differed in their sensitivity to antibiotics. It is interesting to note that strain No. 56 was resistant to ceftazidime, retaining sensitivity to cefepime and cefotaxime, although in the course of previous work we found that all ESBL-producing strains of *K. pneumoniae* isolated between 01.01.2020 and 31.12.2021 were resistant to cefotaxime and possessed the bla_{CTX-M} gene [26].

The strains included in this study belonged to different sequence types, capsule variants, and had different sets of genes for virulence factors and resistance to antibacterial drugs. However, they were united by the presence of genetic determinants *fimH*, *mrkA* and *iutA*. The *fimH* and *mrkA* genes are associated with increased ability to attach to substrates, while the *iutA* gene is associated with the transport of aerobactin [1]. All strains had the bla_{SHV} gene, peculiar to *K. pneumoniae* as a species, providing natural resistance to ampicillin. Mutations in the gene change the substrate specificity and contribute to the inactivation of a wider range of antibacterial drugs. The alleles bla_{SHV-11}, bla_{SHV-1}, bla_{SHV-90} detected in the strains under study are widespread in Russia and were found in strains isolated in Moscow in 2012-2016 [27]. The bla_{SHV-90} gene identified in strain ST23, as well as the *fosA*, *oqxA*, and *oqxB* genes, are similar to the genetic determinants characterized in the vast majority of strains isolated in China and causing both nosocomial and out-of-hospital infections [28].

K. pneumoniae strains with mucus hyperproduction, which have great pathogenic and epidemiologic potential, are currently an urgent problem for the health care system of many countries, so timely detection of such bacterial variants is very important for decision-making on patient management tactics and implementation of anti-epidemiologic measures [29].

Both classical strains producing ESBL and an isolate with a hypermucoid phenotype belonging to the epidemiologically significant hypervirulent ST23 were isolated from newborns of the perinatal center. *K. pneumoniae* ST23 is isolated predominantly in Asia, including Taiwan, Singapore, and mainland China [28], and it is with it that the first descriptions of hypervirulent *Klebsiellae* are associated. ST23 strains of *K. pneumoniae*, which are well studied and frequently encountered in Russia [6], continue to circulate among the population and can colonize the intestine of a newborn child without clinical manifestations of the infectious process. This fact indicates that in the perinatal center, as well as in other medical institutions providing medical care at the inpatient stage, there is still a risk of convergence of hypervirulence and multidrug resistance properties [30], which is a highly undesirable phenomenon due to the emergence of difficulties with the treatment of invasive infections and the need to choose antibacterial drugs from the reserve group for eradication therapy.

During the period over 6 months preceding and following the date of detection of the strain with the hypermucoid phenotype, no isolates with similar phenotypic characteristics were detected in samples from patients and washes from objects in the hospital environment during industrial microbiological control. Taking this into account, it can be concluded that the strain isolated from the fecal sample was an out-of-hospital strain. Conducted local microbiological monitoring and analysis of the data obtained with its help allow timely detection and prevention of joint stay of patients isolating strains with hyperproduction of mucus and resistant to antibacterial drugs, thus preventing cross-infection of patients and undesirable events of microorganism variability that could be realized when they cross paths in one macroorganism.

Currently, there is no unified system of registration and surveillance of circulation of strains with hypermucoviscous phenotype of *K. pneumoniae*. At the same time, their phenotypic detection is realized in wide diagnostic practice of microbiological service during work with bacterial colonies on dense plate nutrient media of various purposes, for example, Endo medium and blood-serum agar. Perhaps, this is due to the fact that hypervirulence should not be identified with hyperproduction of mucus, and the question of choosing the most informative marker of virulence of *K. pneumoniae* remains open to date [20].

Three strains of *K. pneumoniae* researched in the present study, two of which were isolated from a positive hemoculture during generalized infection and the third from a fecal sample during colonization of the intestine of a newborn child, phenotypically manifesting hyperproduction of mucus and possessing the widest spectrum of virulence factor genes, had the same genetic determinants *fimH*, *mrkA* associated with biofilm-forming ability and synthesis of type I and III fimbriae. It is interesting to note that the *traT* gene, which provides serum resistance, was detected in strains isolated from blood samples and was not detected in the isolate from feces, which may have prevented it from overcoming the submucosal layer of the intestinal wall and prevented generalization of the infectious process.

Thus, for the first time in Russia, the results of comparative genomic analysis of clinical isolates of *K. pneumoniae* isolated from newborn infants with different outcomes of the infectious process in the neonatal period are presented, and the well-studied and long-standing sequence types and clonal groups, which have been found on all continents, are identified. No convergent or multidrug-resistant strains were identified in the present study. This is favorable for the epidemiological situation. At the same time, it was found that strains with a high virulence index can be detected during local microbiological monitoring in obstetrics facilities.

Conclusion

1. *K. pneumoniae* strains with single or several virulence determinants are found among patients of neonatal hospitals. The pathogenic potential of *K. pneumoniae* ST23 (virulence index 5) with phenotypically manifested mucus hyperproduction was not realized as an infectious process in the organism of a newborn child.

2. *K. pneumoniae* ST14 with a smaller spectrum of virulence genes than ST23 and low antibiotic resistance (virulence index 0, antibiotic resistance 0) caused a complication during the neonatal period of a premature infant with congenital malformations of the CNS and cardiovascular system in the form of late sepsis with subsequent death. This case demonstrates the complexity of predicting infectious complications at the inpatient stage of nursing newborn premature infants, whose intestines are colonized with *K. pneumoniae*, based only on the genetic and phenotypic characteristics of the microorganism and dictates the necessity for a comprehensive assessment of both the bacterial strain and the patient's health status.

3. The results of this study have supplemented the data on the genetic diversity of strains associated with the neonatal period of development of premature newborn infants and demonstrated the need for further study of the patterns of development of complications of infectious genesis caused by opportunistic microorganisms during their colonization of non-sterile loci of the human body.

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