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Genomic features of resistant *Klebsiella pneumonia*, isolated from the bloodstream and cerebrospinal fluid of pediatric hospital patients

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Abstract

Introduction. Carbapenemase-producing *Klebsiella pneumoniae* (CP-Kp), which are international high-risk clones, have become a problem of utmost importance. CP-Kps, adapting to the hospital environment, evolve into convergent pathotypes. Such variants combine traits of two genetic lineages: multidrug resistant (MDR) and hypervirulent. The pathotypes, along with MDR *K. pneumoniae*, pose an exceptional threat to young patients during systemic infection.

The **objective** of this study is the detailed molecular genetic analysis of MDR isolates of *K. pneumoniae* detected during the monitoring of resistant Gram-negative bacteria at the National Medical Research Center for Children's Health in 2014–2021.

Materials and methods. Whole-genome sequencing with a subsequent bioinformatics analysis of eight MDR isolates from the bloodstream and cerebrospinal fluid.

Results. MDR isolates belonged to 4 sublineages (SL): SL307, SL395, SL29 and SL1198. In the genomes of 6 pangrug-resistant (PDR) isolates, genes associated with resistance to all categories of antibiotics recommended for Enterobacteriaceae therapy were identified. Plasmids were present in all genomes. In 6 isolates, plasmids contained heavy metal ion resistance operons in addition to antibiotic resistance genes. Prophages within the plasmids were also involved in the transfer of resistance genes. The ST395 isolate from the cerebrospinal fluid belonged to the convergent pathotype in terms of resistance and virulence. Comparison of genomes within SLs revealed recombination events in the K- and O-locus regions and the Yersiniabactin operon.

Conclusion. Thus, in a sample of resistant *K. pneumoniae* isolated from bloodstream and cerebrospinal fluid, 6 PDR isolates were detected, one of which belongs to the convergent pathotype ST395.

Keywords: *Klebsiella pneumoniae, whole genome sequencing, cabapenem resistance, extended-spectrum beta-lactamases, multidrug resistance, virulence, plasmids, prophages*

Ethics approval. The study was conducted with the informed consent of the patients. The research protocol was approved by the Ethics Committee of the National Medical Research Center for Children's Health (protocol No. 5a, June 2, 2022).

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Оригинальное исследование
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Геномные особенности резистентных изолятов *Klebsiella pneumoniae*, выделенных из кровяного русла и ликвора пациентов детского стационара

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Аннотация

Введение. *Klebsiella pneumoniae*, продуцирующие карбапенемазы и относящиеся к международным клонам особого риска, адаптируясь к условиям стационара, эволюционируют в конвергентные патотипы. Такие варианты сочетают признаки двух генетических линий: множественно резистентной (MDR) и гипервирулентной. Патотипы, наряду с MDR *K. pneumoniae*, при системной инфекции представляют особую угрозу для пациентов раннего возраста.

Цель исследования — подробный молекулярно-генетический анализ MDR-изолятов *K. pneumoniae*, выявленных при мониторинге резистентных грамотрицательных бактерий в НМИЦ здоровья детей в 2014–2021 гг.

Материалы и методы. Проведено полногеномное секвенирование с последующим биоинформационным анализом 8 MDR-изолятов из крови и ликвора.

Результаты. MDR-изоляты относились к 4 сублиниям (SL): SL307, SL395, SL29 и SL1198. В геномах 6 пан-резистентных (PDR) изолятов выявили гены устойчивости ко всем категориям антибиотиков, рекомендованных для терапии *Enterobacteriaceae*. Плазмиды, присутствовавшие во всех геномах, помимо генов антибиотикорезистентности, в 6 изолятах содержали опероны устойчивости к ионам тяжёлых металлов. В переносе генов резистентности участвовали также профаги в составе плазмид. Изолят ST395 из ликвора по показателям резистентности и вирулентности относился к конвергентному патотипу. Сопоставление геномов внутри SLs выявило рекомбинационные события в областях K- и O-локусов и оперона иерсиниабактина.

Заключение. В выборке резистентных изолятов *K. pneumoniae*, выделенных из кровотока и ликвора, обнаружили 6 изолятов PDR, один из которых относится к конвергентному патотипу ST395.

Ключевые слова: *Klebsiella pneumoniae, полногеномное секвенирование, устойчивость к карбапенемам, бета-лактамазы расширенного спектра действия, множественная устойчивость к антибиотикам, вирулентность, плазмиды, профаги*

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Introduction

Klebsiella pneumoniae occupies the leading position among the *Enterobacteriaceae* causing nosocomial infections in Russia [1]. A concerning problem is the growing proportion of carbapenem-resistant (carbapenemase-producing, CP-Kp) *K. pneumoniae* and the identification of isolates with carbapenemase genes of different classes (A, B, D) simultaneously present in their genomes [1]. According to the data of 2020, CP-Kp in Russia belonged to clonal groups (CG) CG395, CG11, CG147, and CG307 [1], which are classified as international high-risk clones. CP-Kp ST395 prevail in bloodstream infections in patients with hematologic malignancies (37.7%) [2], neurosurgery ICU patients (27.1%) [3], causing severe systemic infections [4]. During the years 2013 to 2018, the AMRmap — national antibiotic resistance monitoring system (https://AMRmap.ru) — identified 108 CP-Kp ST395 isolates included in an international genomic study that showed the emergence of a convergent pathotype in CG395, combining markers of resistance and hypervirulence [5]. Convergence of traits of two genetic lineages of *K. pneumonia*, being multidrug resistant (MDR) and hypervirulent (hvKp), was first reported by Gu et al. for isolate ST11 [6]. Starkova et al. studied convergent (CP- hvKp) *K. pneumonia* isolates ST15, ST147, ST395, ST874, identified in clinics of St. Petersburg, and showed the presence of hybrid plasmids carrying virulence genes and NDM β-lactamase within them [7]. Along with plasmids, other mobile genetic elements such as phages, integrons and transposons contribute to the evolution of bacterial genomes [8]. Conjugative transposons (integrative conjugative elements, ICE) play a specific role in horizontal gene transfer in *K. pneumoniae* [9]. Such a high rate of adaptation and evolution of *K. pneumoniae* in hospital settings poses a particular threat to young patients in need of surgical intervention. Bloodstream infections in such patients lead to unfavorable clinical prognosis [10]. The objective of our study was to analyze MDR *K. pneumoniae* isolated from the bloodstream and cerebrospinal fluid of patients at the National Medical Research Center (NMRC) for Children's Health in 2014–2021.

Materials and methods

Materials

8 cultures of *K. pneumoniae* (**Table 1**) isolated from the bloodstream and cerebrospinal fluid at the NMRC for Children's Health and the Research Institute of Emergency Pediatric Surgery and Traumatology during monitoring of drug resistance of nosocomial isolates of *K. pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* (KAP) in 2014-2021. The study was carried out with the voluntary informed consent of patients and their legal representatives, the study protocol was approved by the Local Ethics Committee

Table 1. Characteristics of antibiotic-resistant *K. pneumoniae* isolates

Patient	Isolate	Data of isolation	Accession number	Genotype	Sublineage	Clonal group (CG)	*wzi* allele	Associated KL type	O locus	O type	Virulence score	Resistance scores
P9	SCCH70:Kpn76815	19.06.2017	JAUPHS000000000	ST307	SL307	CG307	–	KL102*	O1/O2v2	O2afg	1	3
P11	SCCH72:Kpn881723	20.09.2018	JAUPHU000000000	ST2975	SL307	CG307	173	KL102	OL102	OL102	1	3
P8	SCCH69:Kpn69138	13.04.2016	JAUOCC000000000	ST395	SL395	CG395	2	KL2	O1/O2v2	O2afg	4	3
P18	SCCH87:Kpn2182174	05.11.2021	CP122350-CP122355	ST395	SL395	CG395	2	KL2	O1/O2v1	O1	1	3
P17	SCCH84:Kpn2082401	12.09.2020	JAUTIO000000000	ST866	SL1198	CG1198	478	KL46	O3b	O3b	1	3
P17	SCCH86:Kpn207262	02.10.2020	JAUTIQ000000000	ST866	SL1198	CG1198	478	KL46	O3b	O3b	1	3
P10	SCCH71:Kpn863165	17.12.2018	JAUPHT000000000	ST985	SL29	CG985	39	KL39	O1/O2v2	O1	1	3
P12	SCCH73:Kpn96857	11.03.2019	JAUPHV000000000	ST29	SL29	CG29	19	KL19	O1/O2v2	O1	1	3

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of the NMRC for Children's Health (protocol No. 5a dated 02.06.2022). The cultures were characterized by phenotypic multidrug resistance (MDR), extensive drug resistance (XDR) or pandrug Resistance (PDR) in relation to the antibiotic categories recommended by EUCAST for Enterobacteriaceae [11,12], and according to the EUCAST criteria [11].

Methods

Cultivation of *K. pneumoniae* was carried out on blood and Uri-select agar (BioRad) at 37ºC for 24-48 hours. MALDI-TOF mass spectrometer (Bruker Daltonics) was used for identification.

Minimum inhibitory concentrations (MICs) were determined by microdilution for the following categories of antimicrobials:

1) Aminoglycosides: gentamicin, tobramycin, amikacin, netilmicin;

2) Carbapenems: imipenem, meropenem;

3) Extended-spectrum (3rd and 4th generation) cephalosporins: cephalosporin, cefepime;

4) Fluoroquinolones: ciprofloxacin, levofloxacin;

5) Folate metabolism inhibitors: trimethoprim/sulfamethoxazole;

6) Monobactams: aztreonam;

7) Phosphonic acids: fosfomycin;

8) Polymyxins: colistin (polymyxin E), polymyxin B;

9) Penicillins with beta-lactamase inhibitors: ticarcillin/clavulanate, piperacillin/tazobactam.

To identify resistance mechanisms and analyze virulence factors, 8 isolates were examined by whole-genome sequencing.

DNA was extracted from isolates using a method described elsewhere [13], supplemented by purification from polysaccharides using CTAB (cetyltrimethylammonium bromide).

DNA libraries were prepared according to the protocol of Nextera DNA Flex Library Prep (Illumina) and sequenced on a NextSeq 500/550 instrument (Illumina) using a "Mid Output 300 cycles" cartridge.

CLC Genomic Workbench v.21.0.1 (Qiagen) and SPAdes v3.13.0[1] were used for genome assembly. CGView Server was used to visualize the results of replicon assembly and genome comparisons[2] [14]. Annotation was performed using Rapid Annotations Subsystems Technology [15] and NCBI Prokaryotic Genome Annotation Pipeline [16]. PHASTER was used to search for prophage areas[3] [17]. Results of full-genome sequencing were deposited in GenBank in bioproject PRJNA561493 under the numbers shown in Table 1.

Genomic data were analyzed using the resources of the platform Pathogenwatch v. 21.0.0[4], which allows interaction with Kleborate v. 2.2.0[5] [18], a resource specifically designed for *K. pneumoniae* complex research. Kleborate v. 2.2.0 was used to determine genotype in the context of MLST (MultiLocus Sequence Typing) genes, virulence factors: yersiniabactin (*ybt*), aerobactin (*iuc*), colibactin (*clb*), salmohelin (*iro*), hypermucoidity (*rmpA*, *rmpA2*), as well as antimicrobial resistance determinants. With reference to the Kaptive site[6] [19] alleles of the *wzi* gene were determined, and K loci (capsule) and O loci (lipopolysaccharide) were typed.

The criteria developed by Kleborate v. 2.2.0 were used to assess the level of virulence and resistance.

For virulence:

- level 5 — *ybt, iuc, clb*;
- level 4 — *ybt, iuc*;
- level 3 — *iuc*;
- level 2 — *ybt, clb* (or only *clb*);
- level 1 — *ybt*;
- level 0 — complete absence.

For resistance:

- level 3 — carbapenemases and colistin resistance;
- level 2 — carbapenemases;
- level 1 — extended-spectrum β-lactamases without carbapenemases;
- level 0 — absence of extended-spectrum β-lactamases [18].

The VFDB[7] database was used to analyze the extended spectrum of virulence factors [20]. The web platform BIGSdb-Pasteur[8] was accessed to clarify genotypes for *ybt*, *iuc*, *clb*, as well as for the identification of heavy metal ion resistance loci.

A more complete spectrum of resistance genes was determined using the CARD resource[9] [21], as well as the BV-BRC[10], formed on the basis of PATRIC [22].

PlasmidFinder 2.1[11] [23] was used to identify incompatibility groups (Inc) of plasmid replicons.

Results

685 cultures were isolated from bloodstream and cerebrospinal fluid during monitoring of drug resistance of nosocomial isolates of *K. pneumoniae*, *Acineto-*

[1] St. Petersburg genome assembler, Russia, URL: http://cab.spbu.ru/software/spades/
[2] CGView Server. URL: http://stothard.afns.ualberta.ca/cgview_server/
[3] PHAge Search Tool Enhanced Release. URL: https://phaster.ca/
[4] Pathogenwatch v. 21.0.0. URL: https://pathogen.watch/
[5] Kleborate v. 2.2.0. URL: https://github.com/klebgenomics/Kleborate/wiki
[6] Kaptive. URL: https://kaptive-web.erc.monash.edu/
[7] Virulence factor database. URL: http://www.mgc.ac.cn/VFs/
[8] Institut Pasteur Klebsiella pneumoniae species complex. URL: https://bigsdb.pasteur.fr/klebsiella/
[9] Comprehensive Antibiotic Resistance Database. URL: https://card.mcmaster.ca/
[10] Bacterial and Viral Bioinformatics Resource Center. URL: https://www.bv-brc.org/
[11] PlasmidFinder 2.1. URL: https://cge.food.dtu.dk/services/PlasmidFinder/

bacter baumannii, *Pseudomonas aeruginosa* (KAP) in two multidisciplinary children's hospitals: NMRC for Children's Health and Research Institute of Emergency Pediatric Surgery and Traumatology, in 2014-2021, including 63 (9.2%) isolates of *K. pneumoniae*. Eight isolates presented in Table 1 were resistant to multiple categories of antibiotics. Isolates from patients P8-P12 and P18 were PDR according to MIC data for all 9 antibiotic categories. Two *K. pneumoniae* isolated 3 weeks apart from patient P17 were examined. Both isolates were sensitive to fluoroquinolones (to levofloxacin at increased exposure) (**Table 2**). The first isolate by time of isolation was also sensitive to folate metabolism inhibitors and even showed an MIC against polymyxin B corresponding to a borderline resistance value. Thus, the former isolate can be recognized as MDR and the latter as XDR.

Out of the 8 isolates, 7 were isolated from the bloodstream and 1 (P8) was isolated from cerebrospinal fluid. Patients, with the exception of P12 (8 years old), were under 1 year of age, predominantly had genetically determined diseases resulting in malformations requiring surgical intervention. P8 had severe combined trauma, P12 had systemic vasculitis accompanied by hemolytic-uremic syndrome. Fatal outcome was recorded in 2 cases (P10 and P12).

The main characteristics of resistant isolates. All 8 isolates belonged to phylogroup Kp1 and were subdivided into 4 sublineages (SL) (Table 1), 2 of which (307 and 395) are among the most represented in nosocomial *K. pneumoniae* in Russia [1]. Three sublineages included one CG each. SL29 was represented by 2 CGs. Despite the fact that ST985 differs by one locus (*gapA*) allele profile from ST29 (single locus variant, SLV), it belongs to another clonal group — CG985. It is important to note that in CG307, ST2975 is also the SLV of ST307 at the *rpoB* locus.

The SL29 isolates had the same O-antigen variant of O1/O2v2. The same variant was characteristic of the ST307 and ST395 P8 isolates. Another ST395 isolate (P18) had an O-antigen of O1/O2v1. The different variants of the O-antigen are determined by the monomer constituting the polysaccharide. Variant 1 contains d-galactan I and variant 2 contains d-galactan III [24]. Thus, even the isolate genomes of the same genotype may differ in the structure of the operon responsible for the O-antigen. P17 isolates have their own variant, O3b, but similar to the O1/O2 variants, it is prevalent in isolates causing human diseases [24]. Isolate ST2975 has a new O-antigen, therefore it is named OL102 after the capsular antigen number.

In contrast, capsular polysaccharides are distinct in SL29 isolates but identical in ST395 isolates. SL307 isolates have a new KL-type, KL102. Furthermore, while the capsular operon in isolate ST2975 is fully represented, the ST307 genome contains only the full-length *cpsACP* among the genes at the 5'-end of the operon (*galF*, *cpsACP*, *wzi*, *wza*, *wzb*, *wzc*). The *galF* gene is a pseudogene, and 4 other genes, as well as the following *wbaP*, are missing. The *wbaP* gene encodes a glycosyltransferase that initiates capsular polysaccharide synthesis by transferring galactose-1-phosphate to the acceptor udeprenyl-phosphate. In the absence of this enzyme, capsule synthesis is not possible [25]. The wzi and wza genes are responsible for outer membrane channel proteins, while the wzc and wzb genes are regulators of polymerization and transport of capsular polysaccharides to the surface of the bacterial cell.

The fimbriae operons fimA–fimK (type 1) and mrkA–mrkJ (type 3), as well as the type IV pilW pilus gene were present in the genomes of all isolates.

In order to determine the resistance and virulence of the selected isolates based on genomic data, we used the quantitative assessments offered by Kleborate v. 2.2.0 [19] (Table 1). The genomes of all isolates contained the *ybt* operon, which is located in ICE*Kp*: ICE*Kp*4 (ybt10) in CG307 isolates, ICE*Kp*5 (ybt14) in ST395 P8 and ST29 isolates, ICE*Kp*12 (ybt16) in ST395 P18 and ST985 isolates, and ICE*Kp*15 (ybt18) in ST866 isolates.

Most of the isolates had a virulence level of 1. The ST395 P8 isolate had a level of 4, the highest, because in addition to *ybt*, the isolate's genome encodes aerobactin (AbST95). Of the additional virulence genes,

Table 2. Comparison of antibiograms of *K. pneumoniae* ST866 isolates from patient P17 in three categories of antibiotics

Isolate	MIC, mg/L			
	fluoroquinolones		folate pathway inhibitors	polymyxins
	ciprofloxacin	levofloxacin	trimethoprim/sulfamethoxazole	Polymixin B
SCCH84:Kpn2082401	≤ 0,25	1*	2	2
SCCH86:Kpn207262	0,5*	1*	16/304	16
Resistance — MIC breakpoints, mg/L	0,5	1	4	2

Note. *The sensitivity at increased exposure; the cells corresponding to the sensitivity of the isolate to the antimicrobial drug are highlighted in grey.

peg-344 [26], responsible for drug and metabolite transporter permease, is present only in the ST395 P8 genome. The hypermucoidity genes *rmpA* and *rmpA2* were not detected in this group of isolates.

The resistance level is the highest in all isolates (3), because both carbapenemase genes and mutations in genes (*PhoP_26Q*) providing resistance to colistin (polymyxin E) are present in the genomes [27].

The spectrum of **β-lactamase genes** in the genomes of isolates is extensive (**Fig. 1**). All genomes contain at least one carbapenemase: OXA-48 (class D) or NDM-1 (class B, metallo-β-lactamase), and the ST2975 and ST395 P18 genomes contain both β-lactamases. NDM-1 is also classified as an extended-spectrum β-lactamase [28], to which CTX-M-15 and CTX-M-3 (class A) also belong. Only the ST395 P18 isolate did not contain CTX-M lactamase. As far as the other class A β-lactamases is concerned, the TEM and SHV lactamases are present in all genomes. The CMY-6 cephalosporinase (class C) is present in most genomes; it is absent only in the ST307 and ST395 P8 genomes. However, these genomes have an additional class D β-lactamase, OXA-1, which is also present in the ST985 genome. The ST29 genome is distinguished by the presence of two additional class D β-lactamases, OXA-10 and OXA-244. Thus, the isolate genomes contain at least 5 genes of β-lactamases of different classes, while there are 7 genes in the ST29 genome.

Resistance genes to other antibiotic categories are shown in **Fig. 2.** A minimum of 6 additional resistance genes were detected in ST866 isolates. Among them there are no resistance genes to fluoroquinolones and trimethoprim, which confirms the phenotypic data. The maximum number of additional genes in isolate ST395 P8 is 16. Along with isolate ST985 (14 genes), they have at least one resistance-determining gene for each category of antibiotics. It is important to note that only the leading isolates have macrolide resistance genes.

Heavy metal ion resistance genes were found in 6 out of 8 genomes (**Fig. 3**). These are resistance operons to arsenic (*arsABCDR*), copper (*pcoABCDERS*), silver (*silABCEFGPRS*), and tellurium (*terABCDEWXYZ*). All 4 operons are present in the ST307 genome, 3 each in the ST2975 and ST985 genomes, 2 each in ST866, with only the tellurium resistance operon being present in the ST395 P8 genome.

Plasmids of resistant isolates. Typically, heavy metal operons and many resistance genes are present in plasmids [29]. We estimated the number of Inc plasmid groups for the genomic data of each isolate (**Fig. 4**). The maximum number of Inc was found in isolate ST307 (7) and the minimum in isolates ST985 (1) and ST866 (3). The data confirm the presence of plasmids in the genomes of all isolates.

The different spectrum of Inc in isolates of the same ST395 genotype attracts attention. Assembly of

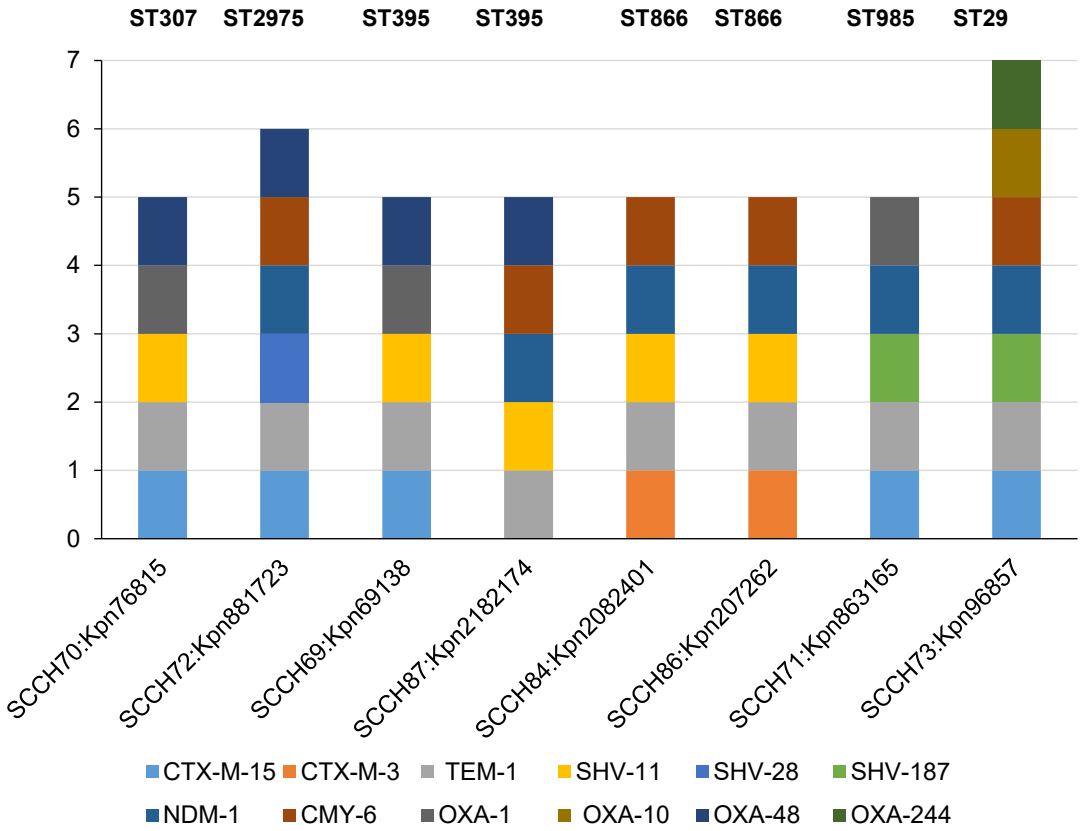

Fig. 1. Diversity of beta-lactamase genes in the genomes of the studied isolates.

plasmid replicons of ST395 P8 and ST395 P18 isolates showed that the ST395 P18 genome contains 5 plasmids, 3 of which are capable of conjugative transfer, and the ST395 P8 genome contains 4 plasmids, one of which is cryptic (2 are capable of conjugative transfer). The plasmidome of the isolates differed in the number and set of resistance and virulence genes (**Fig. 5**). The plasmidome of ST395 P8 was characterized by

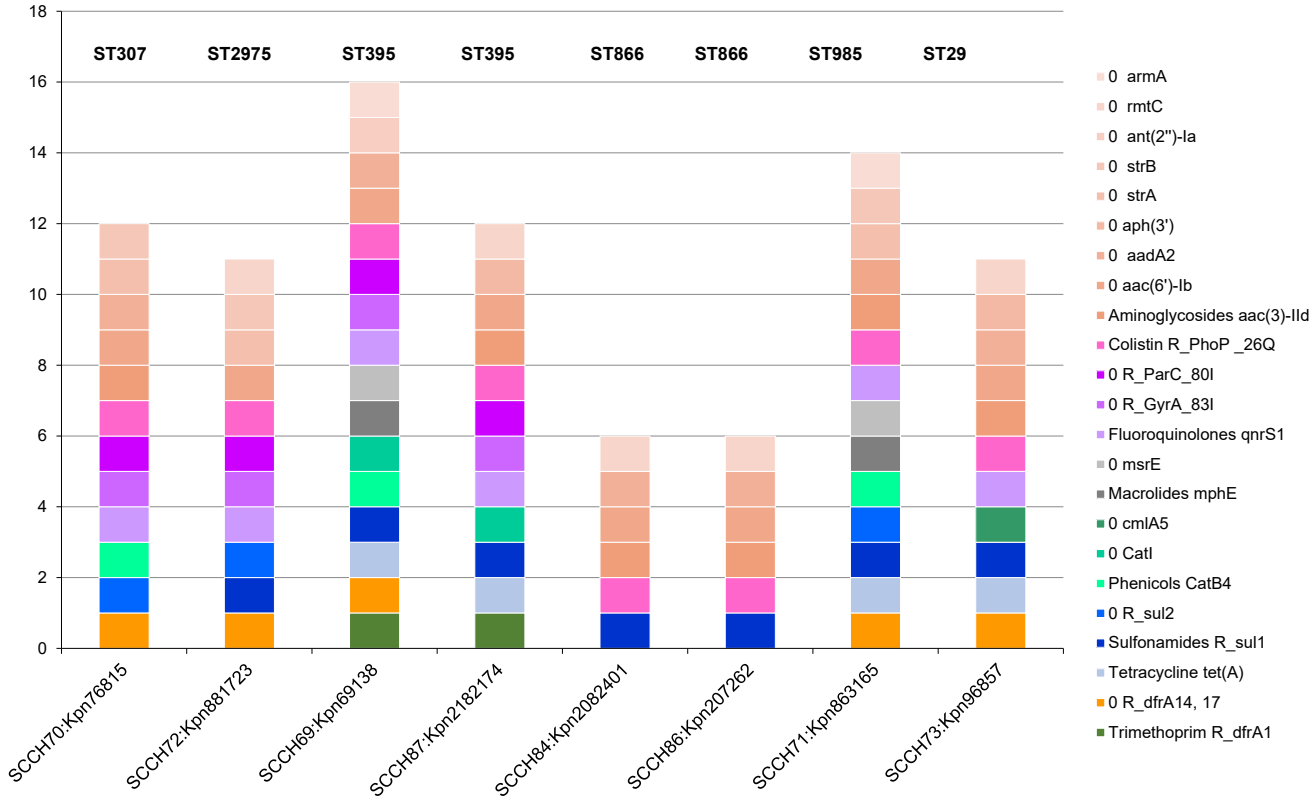

Fig. 2. Diversity of resistance genes to other categories of antibiotics in the genomes of the studied isolates.

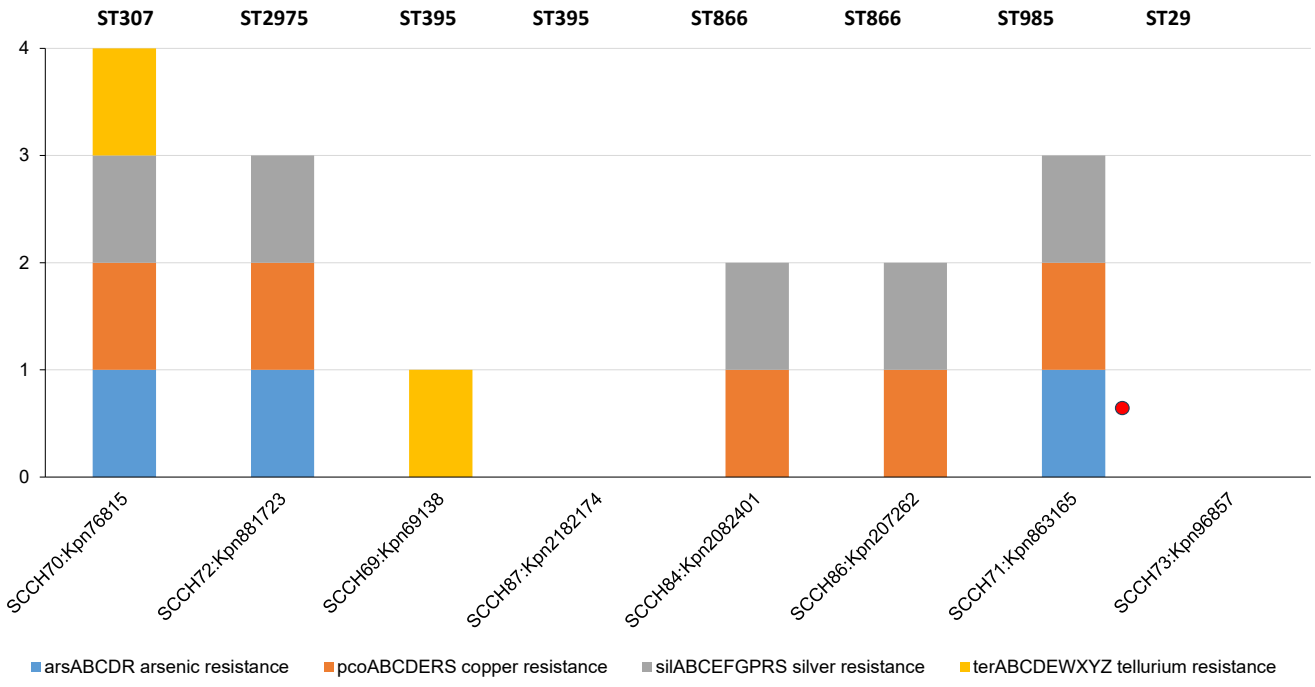

Fig. 3. Genes of resistance to heavy metal ions in the genomes of the studied isolates.
The red dot marks the incomplete *arsBCRD* operon.

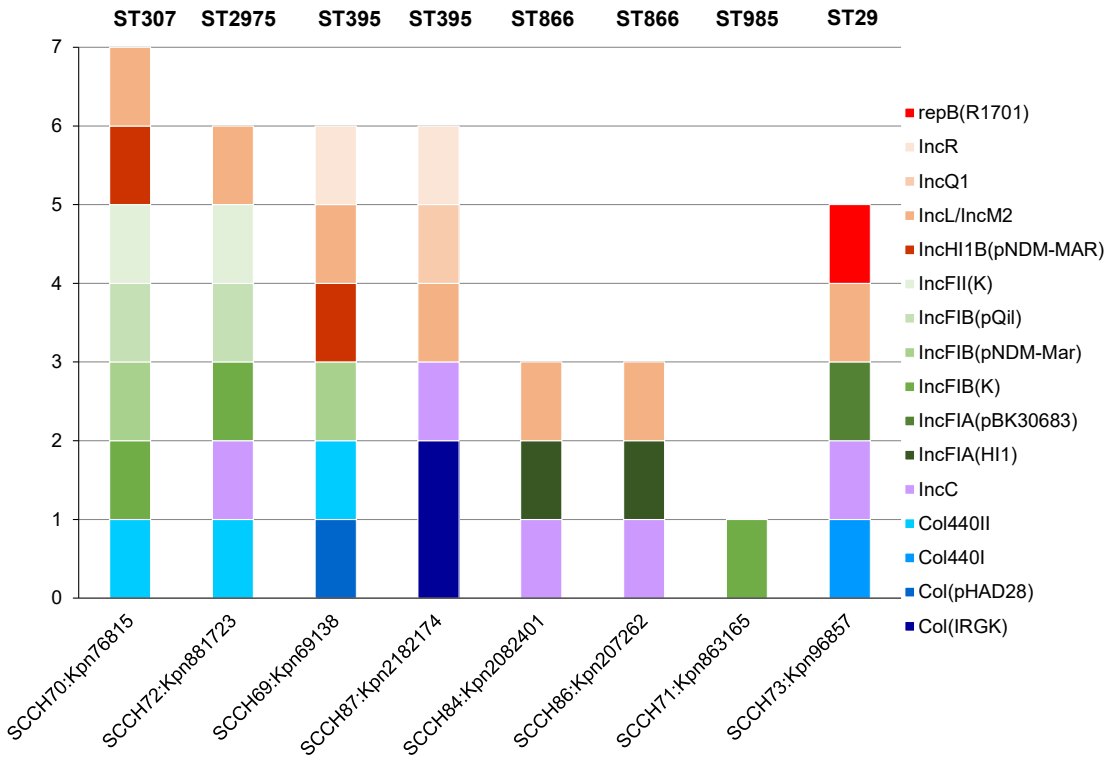

Fig. 4. Diversity of origins of replication (Inc) plasmids in the studied isolates.

the presence of extended spectrum β-lactamase genes, aerobactin, *peg-344*, tellurium resistance operon, and a greater number of β-lactamase genes. The plasmidome of ST395 P18 had more carbapenemase genes, sulfonamide and aminoglycoside resistance genes. Thus, the plasmidome of ST395 isolates underwent significant changes from 2016 to 2021.

Comparison of the prophages of ST395 isolates showed differences in these mobile genetic elements as well. Eight prophages were identified in the ST395

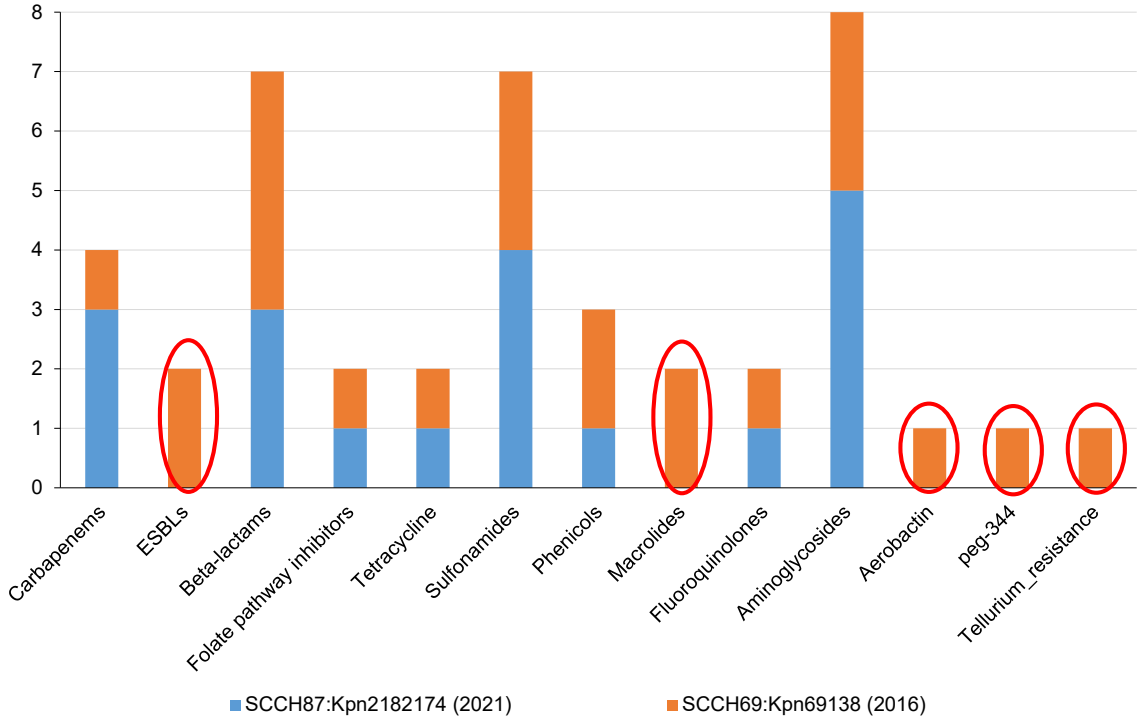

Fig. 5. Resistance and virulence genes in *K. pneumoniae* ST395 plasmids.
Circled are factors that are present only in the plasmids of the ST395 P8 isolate.

P8 genome, 7 in the ST395 P18 genome; 5 prophages were common to the ST395 genomes. However, ST395 P8 plasmids contained twice as many prophages (10) compared to ST395 P18 plasmids (5). All prophages had homology with 17 different bacterial phages of the *Caudoviricetes* class. The chromosome prophages each contained 3 holin genes that form pores in the cell membrane [30], and lysin genes that damage peptidoglycan: 4 lysozyme genes, ST395 P8, and 5 lysozyme genes and a membrane-bound lytic transglycosylase D gene, ST395 P18. CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats) systems were absent in both genomes. At the same time, the analysis of prophage plasmids of ST395 isolates confirmed the observation that phages could possibly be carriers of antibiotic resistance genes [31]. The prophages of plasmids of ST395 P8 isolate contained genes of β-lactamases (5), chloramphenicol acetyltransferases (3), aminoglycoside acetyltransferases (2), dihydrofolate reductase, streptomycin adenyltransferase, and tellurium resistance operon. The plasmid prophages of isolate ST395 P18, which contained genes for aminoglycoside acetyltransferases (3), dihydropteroate synthases (2), β-lactamases TEM and OXA-48, chloramphenicol acetyltransferase and 3 SMR (Small multidrug resistance) efflux transporters, were not inferior to them either.

Discussion

The study revealed a great diversity of MDR *K. pneumoniae* genotypes isolated from cerebrospinal fluid and bloodstream. According to the data of the Pasteur Institute[12], ST307 is the most prevalent worldwide (1786 isolates, 21 in Russia), followed by ST395 (380, 188 in Russia) and ST29 (255, 2 in Russia), ST985 (48), ST2975 (29, 12 in Russia), and ST866 (7). Infection with PDR *K. pneumoniae* ST29 and ST985 resulted in fatal outcomes. The systemic infection was detected in other loci besides the bloodstream in both patients P10, P12 and patients P9, P11, P17, P18. The infection in Patient P8 was detected only in the cerebrospinal fluid and was caused by the most virulent ST395 isolate (level 4). In terms of resistance (PDR) and virulence, the ST395 P8 isolate matched the characteristics of the convergent ST395 pathotype [5]. The study of the isolate plasmids confirms the transfer of resistance factors (including to heavy metal ions) and virulence factors by these mobile genetic elements and prophages within the plasmids. Comparison of the ST395 P8 and ST395 P18 genomes reveals evidence of recombination in hot spots characterized by E.R. Shaidullina et al. [5]. The O-antigen, ICE*Kp* and prophage regions differ among isolates. Isolate ST395 P18 has ICE*Kp*12 (ybt16) prevalent in CG305 and in subclade B1 (KL2KL30) [5], with the K-locus characterization of the isolate corresponding to that of the subclade. In the ST395 P8 isolate, ICE*Kp*5 (ybt14) is rare [5] and belongs to subclade A1 (KL108). However, the ST395 P8 isolate contains a KL2 K-locus, which may be further evidence of recombination events.

In CG307 isolates, differences in the set of resistance genes are associated with a different plasmid profile. In the chromosome, the isolates contain the same ICE*Kp*4 (ybt10) regions, but significant differences in the main recombination hot spot, the K- and O-loci.

In SL29, the isolates are similar only in the O-locus, have different K-loci, ICE*Kp*, plasmid profile and resistance gene spectrum. It is important to note that the single plasmid of isolate ST985 (IncFIB(K)) contains 3 heavy metal ion resistance operons and a large list of genes that define together with genes of the chromosome pandrug-resistance of the isolate.

ST866 isolates, while being the least resistant, are nevertheless MDR and XDR, carrying plasmids containing 2 operons of resistance to heavy metal ions.

Returning to the actual problem of resistance of *K. pneumoniae* to carbapenems, it is necessary to note the presence of 2 isolates containing 2 carbapenemase genes each: OXA-48 and NDM-1, in the sample. The isolates belong to international high-risk clones CG395 and CG307.

Thus, in the sample of resistant isolates of *K. pneumoniae* isolated from bloodstream and cerebrospinal fluid, 6 PDR isolates were detected, one of which belongs to the convergent pathotype ST395.

[12] Institut Pasteur. URL: bigsdb.pasteur.fr/klebsiella

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