MICROBIOLOGY AND VIROLOGY

BIOLOGICAL PROPERTIES AND GENETIC STRUCTURE OF CLINIC ISOLATES OF *KLEBSIELLA PNEUMONIAE* SPECIES

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ABSTRACT

Klebsiella pneumoniae (Kp) species complex is a genetically and ecologically diverse group of bacteria that causes a wide range of infections in humans and animals.
The aim of the study. *To carry out biological characterization and genotyping based on the study of different loci of Klebsiella pneumoniae clinical isolates.*
Materials and methods. *The object of the study was three Klebsiella pneumoniae clinical isolates from different biotopes of patients from a regional children's multidisciplinary hospital. We used a complex of bacteriological, molecular genetic and bioinformatic methods. Genotyping of the isolates was carried out using the Pasteur Institute service for strains of the K. pneumoniae species complex.*
Results. *All strains were susceptible to antimicrobial drugs from carbapenem (imipenem, meropenem) and tetracycline groups (tigecycline), and demonstrated high susceptibility to the Klebsiella polyvalent bacteriophage. The antibiotic resistance of the Kp ODKB-16 and ODKB-81 isolates to seven and eight antimicrobial drugs, respectively, was registered.*
Based on the results of multilocus sequence typing, all strains were assigned to Kp1 phylogroup, K2 type and differed in sequence type, scgMLST629 profile, and KL type. Kp ODKB-16 strain was identified as ST-65, scgST-11107, KL2; ODKB-07 strain – as ST-219, scgST-6401, KL125KL114; ODKB-81 strain – as ST-86, scgST-2800, KL2KL30. The virulence gene clusters AbST, CbST, YbST, SmST, and RmST have been characterized only in the genome of the Kp ODKB-16 isolate, allowing it to be characterized as highly virulent with multidrug resistance (MDR). Additionally, genes responsible for the synthesis of types 1 and 3 fimbrial adhesins were registered in all strains, and ter operon loci were identified only in Kp ODKB-16. Resistome analysis showed that all strains had 2b genotype. Plasmids were found in the genomes of Kp ODKB-81 (IncI2) and ODKB-16 (IncFIA + IncFIB + IncHI1B).
Conclusion. *We used a comprehensive framework for genomic taxonomy of clinical isolates, which can contribute to the unification of global and regional peculiarities of the developing and microevolution of bacterial pathogens.*

Key words: *Klebsiella pneumoniae species complex, genotyping, multilocus analysis, multiple antibiotic resistance, antimicrobial drugs, virulence*

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БИОЛОГИЧЕСКИЕ СВОЙСТВА И ГЕНЕТИЧЕСКАЯ СТРУКТУРА КЛИНИЧЕСКИХ ИЗОЛЯТОВ КОМПЛЕКСА ВИДОВ *KLEBSIELLA PNEUMONIAE*

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РЕЗЮМЕ

Комплекс видов Klebsiella pneumoniae (Kp) представляет собой генетически и экологически разнообразную группу бактерий, вызывающую широкий спектр инфекций у людей и животных.

Цель исследования. *Биологическая характеристика и генотипирование на основе изучения разных локусов клинических изолятов Klebsiella pneumoniae.*

Материалы и методы. *Объектом исследования стали три клинических изолята Kp, выделенные из разных биотопов пациентов детского многопрофильного стационара регионального уровня. В работе использован комплекс бактериологических, молекулярно-генетических и биоинформационных методов. Генотипирование изолятов проводили с использованием сервиса Института Пастера для штаммов видового комплекса K. pneumoniae.*

Результаты. *Все штаммы были чувствительны к антимикробным препаратам групп карбапенемы (имипенем, меропенем) и тетрациклины (тигециклин) и демонстрировали высокую чувствительность к бактериофагу клебсиелл поливалентный. У изолятов Kp ODKB-16 и ODKB-81 отмечена антибиотикорезистентность к семи и восьми антимикробным препаратам соответственно.*

Согласно результатам мультилокусного типирования, все штаммы отнесены к филогруппе Kp1, имели K2-тип и различались по сиквенс-типам, профилю scgMLST629 и KL-типу. Штамм Kp ODKB-16 был определён как ST-65, scgST-11107, KL2; ODKB-07 – как ST-219, scgST-6401, KL125KL114; ODKB-81 – как ST-86, scgST-2800, KL2KL30. Кластеры генов вирулентности AbST, CbST, YbST, SmST и RmST были охарактеризованы только в геноме изолята Kp ODKB-16, что позволяет охарактеризовать его как высоковирулентный с множественной лекарственной устойчивостью (МЛУ). Дополнительно у всех штаммов выявлены гены, ответственные за синтез фимбриальных адгезинов 1-го и 3-го типов, а локусы ter-оперона – только у Kp ODKB-16. Анализ резистома показал, что все штаммы имели генотип 2b. Плазмиды были определены в геномах Kp ODKB-81 (IncI2) и ODKB-16 (IncFIA + IncFIB + IncHI1B).

Заключение. *Использована комплексная схема для геномной таксономии клинических изолятов, которая может способствовать унификации глобальных и региональных особенностей возникновения и микроэволюции бактериальных патогенов.*

Ключевые слова: *комплекс видов Klebsiella pneumoniae, генотипирование, мультилокусный анализ, множественная антибиотикорезистентность, антимикробные препараты, вирулентность*

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BACKGROUND

The *Klebsiella pneumoniae* species complex is a genetically and ecologically diverse group of bacteria causing a wide range of infections in humans and animals [1]. Considering its diversity, as well as evolutionary dynamics, multidrug resistance and virulence, typing schemes for *K. pneumoniae* strains are being developed [1-3]. Controversial microbial species identification and horizontal gene transfer underlie the extensive strain heterogeneity of their phenotypes, which is of ecological, medical, and/or industrial importance [4]. Obviously, the most successful taxonomic system for genetic typing of microbial strains is the multilocus sequencing approach [5, 6]. This system is used for population biology studies and surveillance of bacterial pathogens in public health [7]. Several genotyping schemes based on different loci have been developed for the *K. pneumoniae* species complex: proper multilocus typing (MLST, multilocus sequence typing) [8], multilocus typing by main genome (cgMLST, core genome multilocus sequence typing) [2, 3], typing by *wzc* and *wzi* genes [9], as well as multilocus typing of virulence gene clusters (AbST, CbST, YbST, SmST, RmST), and antibiotic resistance (aminoglycosides, beta-lactamases, quinolones) [2, 10, 11].

It has been previously revealed that *K. pneumoniae* in 23.1 % of cases acts as an etiological factor in the development of hospital purulent-septic infections in a paediatric multidisciplinary hospital at the regional level [12]. When microbiological data from clinically relevant biotopes (blood, sputum, urine, wound contents, abdominal fluid, tracheobronchial flushes, and liquor) were examined, it was revealed that blood (32.3 %) and sputum (27.1 %) had the highest frequency of microbial isolation [12]. These clinical isolates of *K. pneumoniae* have been experimentally observed to form biofilms with varying efficacy, showing different resistance to disinfectants and antimicrobial agents (AMAs) [13-15]. It may also be further noted that traditional methods of clinical bacteriology do not allow distinguishing strains within the *K. pneumoniae* species complex, which masks the true clinical significance of each sequencing type/phylogroup and their potential epidemiological features [9]. Considering this statement, the isolation biotope and multiple AMA resistance were taken into account when selecting strains for genetic typing.

THE AIM OF THE STUDY

Biological characterisation and genotyping based on different loci of three clinical isolates of *K. pneumoniae* isolated from different biotopes of patients of a regional paediatric multidisciplinary hospital.

METHODS

Three isolates of *K. pneumoniae* were studied from the clinical material of patients being treated in the intensive care unit of a paediatric multidisciplinary hospital at the regional level (Irkutsk).

The isolates were determined according to morphological, tinctorial, culture, and biochemical properties using bioMérieux API systems (France) and confirmed by mass spectrometric analysis with the ultraflExtreme mass spectrometer (Bruker Daltonics, Germany) [16].

To assess susceptibility to AMA, bacterial suspension was prepared according to standard methodology with an optical density of 0.5 McFarland. AMA susceptibility was determined by disc-diffusion method using Mueller – Hinton medium (HiMedia, India); the results were analysed in accordance with the current regulations and interpretation tables of the European Committee on Antimicrobial Susceptibility Testing (EUCAST; version 11.0, valid from January 01, 2021) [17-19]. Strains exhibiting the R criterion were categorised as resistant, I - susceptible with increased exposure, and S – susceptible. The study included discs containing aztreonam (AZT; 30 μg), amikacin (AMK; 30 μg), amoxicillin-clavulanic acid (AMC; 20-10 μg), gentamicin (GEN; 10 μg), imipenem (IPM; 10 μg), meropenem (MER; 10 μg), netilmicin (NET; 10 μg), piperacillin-tazobactam (PIT; 30 μg – 6 μg), tigecycline (TGC; 15 μg), cefepime (CEP; 30 μg), ceftazidime (CAZ; 10 and 30 μg) (NICF LLC, Russia).

To assess the susceptibility of *K. pneumoniae* isolates to phages, a commercial preparation of bacteriophage produced by SPA (Scientific Production Association) «Microgen» (Russia) with declared activity against Klebsiella – Klebsiella polyvalent bacteriophage (20 ml vials, series U387 02.2020; Ufa) was used. Determination of the level of lytic activity (LLA) of bacteriophage to *K. pneumoniae* isolates was performed by the drop method (spot-test) [20, 21].

Whole-genome sequencing was performed on NextSeq 550 (Illumina, USA) equipment using the Illumina DNA Prep Tagmentation, IDT for Illumina DNA/RNA UD Indexes Set Tagmentation, and NextSeq 500/550 High Output Kit v2.5 (300 Cycles) library preparation reagent kits, according to the manufacturer's recommendations. Pre-contig primary data were assembled using SPAdes v. 3.11.1 [22]. Contigs were aligned against the *Klebsiella pneumoniae* subsp. *pneumoniae* HS11286 reference genome (GenBank CP003200) and corrected using MAUVE 2.4.0 (The Darling Lab, Australia) [23]. Prokka 1.14.6 (Oregon State University, USA) was used for functional annotation [24]. MOB-Typer (National Microbiology Laboratory, Canada) was used to characterize plasmids [25]. Mobile genetic elements (MGEs) (IS elements and transposons) were searched using IS-finder (France) [26].

Genotypes of isolates were determined using the Pasteur Institute database for strains of the *K. pneumoniae* species complex [27] based on MLST [8], cgMLST [2, 3], *wzc* and *wzi* gene typing [9], and MLST clusters of virulence genes (AbST, CbST, YbST, SmST, RmST) as well as antibiotic resistance (aminoglycosides, beta-lactamases, quinolones) genes [2, 10, 11].

This study was performed within the framework of the state task No. 121022500179-0 using

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RESULTS

A brief characterization of the analyzed *K. pneumoniae* isolates is presented in Table 1. Based on AMA susceptibility results, all strains showed resistance to more than two AMAs of different groups, but were susceptible to imipenem, meropenem (carbapenem group), and tigecycline (tetracyclines). In addition, all isolates revealed high susceptibility to *Klebsiella* polyvalent bacteriophage (Table 1). It should also be mentioned that *K. pneumoniae* isolates ODKB-16 and ODKB-81 revealed resistance to seven and eight drugs, respectively, and can be defined as multidrug resistant (MDR) strains.

A summary of the genome assembly and annotation results are summarized in Table 2. The genomes of the strains were mapped to the *Klebsiella pneumoniae* subsp. *pneumoniae* HS11286 reference genome and are shown in Figure 1. The genome sizes of the analyzed isolates varied slightly and were 5.7×10^6, 5.3×10^6, and 5.4×10^6 base pairs - b.p. for *K. pneumoniae* ODKB-16, *K. pneumoniae* ODKB-07, and *K. pneumoniae* ODKB-81, respectively. The guanine-cytosine (GC) composition was consistent with the characterization of the *K. pneumoniae* species. Plasmids belonging to different incompatibility groups were revealed in *K. pneumoniae* strains ODKB-16 and *K. pneumoniae* ODKB-81 (Table 3). It is worth noting that *K. pneumoniae* ODKB-81 carries IncI2 incompatibility group plasmid, while *K. pneumoniae* ODKB-16 carries a combined plasmid belonging to several groups simultaneously (IncFIA + IncFIB + IncHI1B), which is a characteristic feature of clinical isolates. Additionally, MOBF-type relaxase was revealed in *K. pneumoniae* ODKB-16 plasmid and conjugative mobility was predicted. Profages and integrons were not found in any isolate. MGEs were represented by insertional IS-elements and Tn-transposons. MGEs of the IS1380 and IS3 families were revealed in all three isolates in the chromosome. In addition, IS66 was revealed in the *K. pneumoniae* ODKB-7 chromosome, and IS5 and IS1 elements were revealed in *K. pneumoniae* ODKB-16. The MGEs of these families are small in length, ranging from 500 to 2000 bp. – and do not contain genes responsible for resistance or pathogenicity. Two MGEs of the IS481 and IS66 families, which do not carry resistance or virulence genes, were revealed in the *K. pneumoniae* ODKB-81 plasmid. 18 MGEs belonging to the IS1, IS3, IS4, IS5, IS6, IS21, IS66, IS110, IS481, IS630, IS1380, ISNCY, and Tn3 families were revealed in the *K. pneumoniae* ODKB-16 plasmid. One Tn3 MGE of the family included genes *pbrR* (transcription factor belonging to the MerR family), *pbrA* (P1B-type ATRase), *pbrB* (integral membrane protein), and *pbrC* (putative signaling peptidase). The *pbrTRABCD* gene cluster is thought to encode a unique, specific mechanism for lead resistance [28].

Genetic typing of *K. pneumoniae* included sequencing-type determination by MLST and cgMLST multilocus sequencing schemes, identification of markers associated with phenotypic capsule serotyping, and search for virulence and antibiotic resistance determinants.

TABLE 1

BRIEF CHARACTERIZATION OF ISOLATES OF THE *K. PNEUMONIAE* SPECIES COMPLEX

Isolate labeling	Isolation source (date)	Antibiotics		Genotype (23 loci*)	Polyvalent Klebsiella bacteriophage
		Susceptibility	Resistance		
K. pneumoniae ODKB-16	Tracheobronchial tree (November 06, 2018)	AMC20-10, IPM10, MER10, TGC15	AMK30, GEN10, NET10, PIT30-6, CEP30, CAZ10, CAZ30, AZT30	2b	3X
K. pneumoniae ODKB-07	Blood (June 21, 2018)	AMK30, GEN10, NET10, AMC20-10, PIT30-6, IPM10, CAZ30, MER10, TGC15	CEP30, CAZ10, AZT30	2b	3X
K. pneumoniae ODKB-81	Sputum (October 22, 2019)	IPM10, MER10, TGC15	AMK30, GEN10, NET10, AMC20-10, PIT30-6, CEP30, CAZ10, CAZ30, AZT30	2b	4X

Note. * – genotyping was performed at the following loci: blaAMPC, blaBEL, blaCARB, blaCMY, blaCTX_M, blaGES, blaIMP, blaIND, blaKPC, blaLEN, blaNDM, blaOKP_ABCD, blaOXA, blaOXY, blaPER, blaSHV, blaSME, blaTEM, blaVEB, blaVIM.

TABLE 2

SUMMARY RESULTS OF GENOME ASSEMBLY AND ANNOTATION OF GENOMES OF ISOLATES OF THE *K. PNEUMONIAE* SPECIES COMPLEX

Characteristics	*K. pneumoniae* ODKB-16	*K. pneumoniae* ODKB-07	*K. pneumoniae* ODKB-81
Genome assembly results			
Number of readings per sample	18 689 678	23 218 431	8 283 075
Scaffold quantity	73	66	61
N50	293 674	308 988	308 386
Genome annotation results			
Genome size, b.p.	5 693 553	5 333 942	5 368 963
GC, %	56.69	57.19	57.33
Number of protein-coding sequences	5 421	5 085	4 892
rRNAs quantity	4	7	6
tRNAs quantity	63	66	54
Plasmid quantity	1	No	1

TABLE 3

BRIEF CHARACTERIZATION OF PLASMIDS OF *K. PNEUMONIAE* SPECIES COMPLEX ISOLATES

Characteristics	*K. pneumoniae* ODKB-16	*K. pneumoniae* ODKB-81
Size, b.p.	359 611	35 021
GC, %	50.37	43.25
Incompatibility groups	IncFIA+IncFIB+IncHI1B, rep_cluster_1254	IncI2
Probable origin	*Klebsiella pneumoniae*	*Escherichia coli*

According to the results of MLST multilocus typing for seven genes *gapA, infB, mdh, pgi, phoE, rpoB,* and *tonB*, all strains showed a different genotype: *K. pneumoniae* ODKB-16 was identified as ST-65, *K. pneumoniae* ODKB-07 as ST-219, *K. pneumoniae* ODKB-81 as ST-86 (Table 4). All strains were assigned to the Kp1 phylogroup, *K. pneumoniae* ODKB-16 and *K. pneumoniae* ODKB-81 were K2-type according to the *wzc* and *wzi* genes, but differed in scgMLST629 profile and KL-type (Table 4).

The virulence profile of *K. pneumoniae* isolates was characterized using the virulence gene clusters AbST, CbST, YbST, SmST, and RmST typing at the Aerobactin, Colibactin, Ersiniabactin, Salmohelin, and RmST/RmpADC protein family loci, respectively. All virulence gene clusters were identified and characterized only in the genome of the *K. pneumoniae* isolate ODKB-16 (Table 5); their localization is presented on the mapped genome (Fig. 16). Thus, only the *K. pneumoniae* isolate ODKB-16 can be characterised as highly virulent with MDR.

Additionally, pathogenicity determinants that are not included in the list of marker genes and their regions that are validated for typing strains of the *K. pneumoniae* species complex were searched in the genomes [27]. It is known that strains of *K. pneumoniae* complex having serotype K2 express invasive properties [9]. Genes responsible for the synthesis of fimbrial adhesins of type 1 and type 3 (*fimA* and *mrkD*, respectively) were found in the chromosome structure of the studied *K. pneumoniae* strains. However, genes responsible for the synthesis of invasins, adhesin-pili P, α-hemolysin, thermolabile enterotoxins, and for the manifestation of the hypermucoid phenotype (*rmpA* and *magA*) were not revealed. A tellurite resistance operon (TeO_3^{-2}, *ter* operon) was searched within the genomes, which

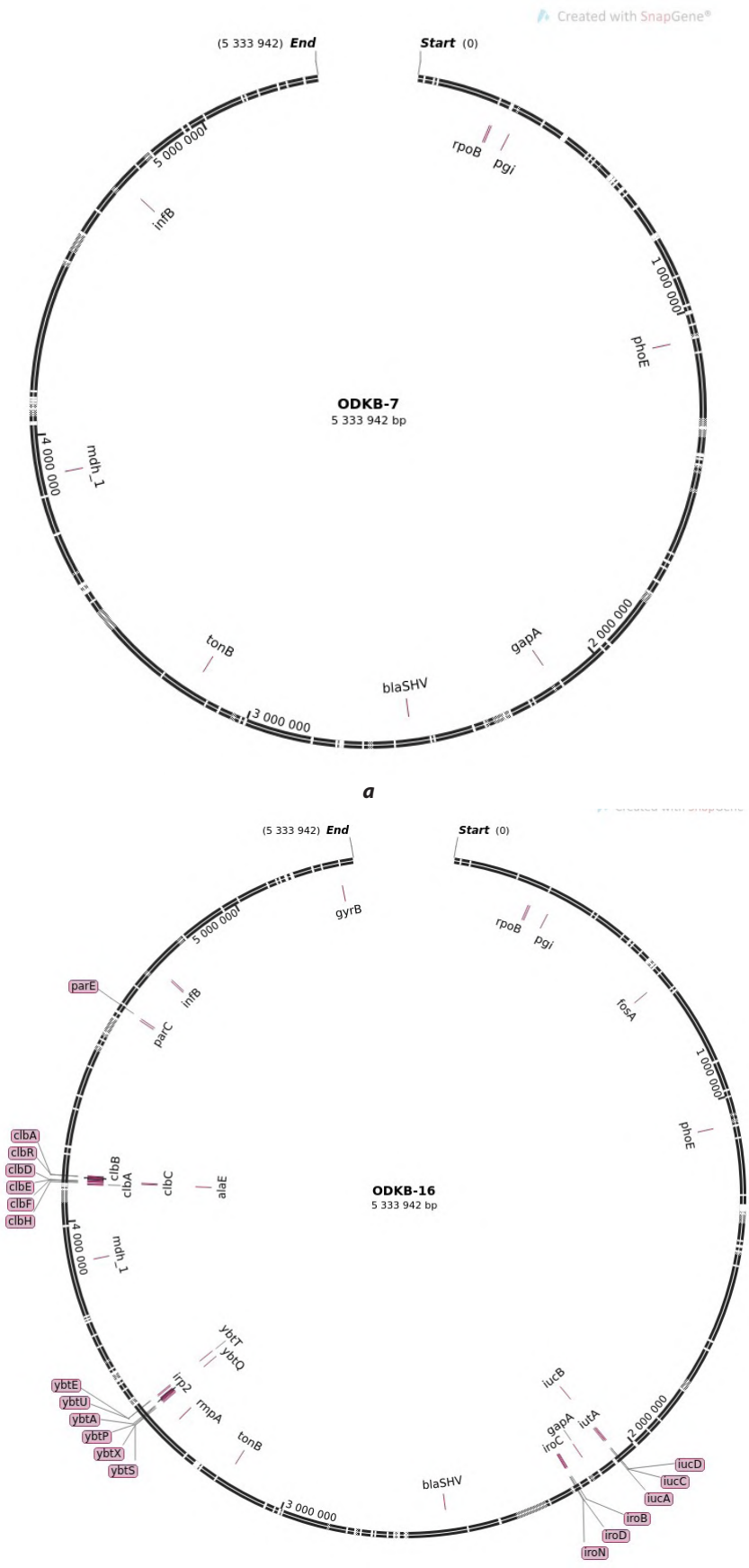

FIG. 1.

Chromosome maps of K. pneumoniae ODKB-7 (а), K. pneumoniae ODKB-16 (б), K. pneumoniae ODKB-81 (в) strains mapped to the K. pneumoniae subsp. pneumoniae HS11286 reference genome. Genes identified by MLST (gapA, infB, mdh, pgi, phoE, rpoB, and tonB) as well as MLST analysis of virulence determinants (AbST, CbST, YbST, SmST, RmST) and antibiotic resistance are indicated in the footnotes

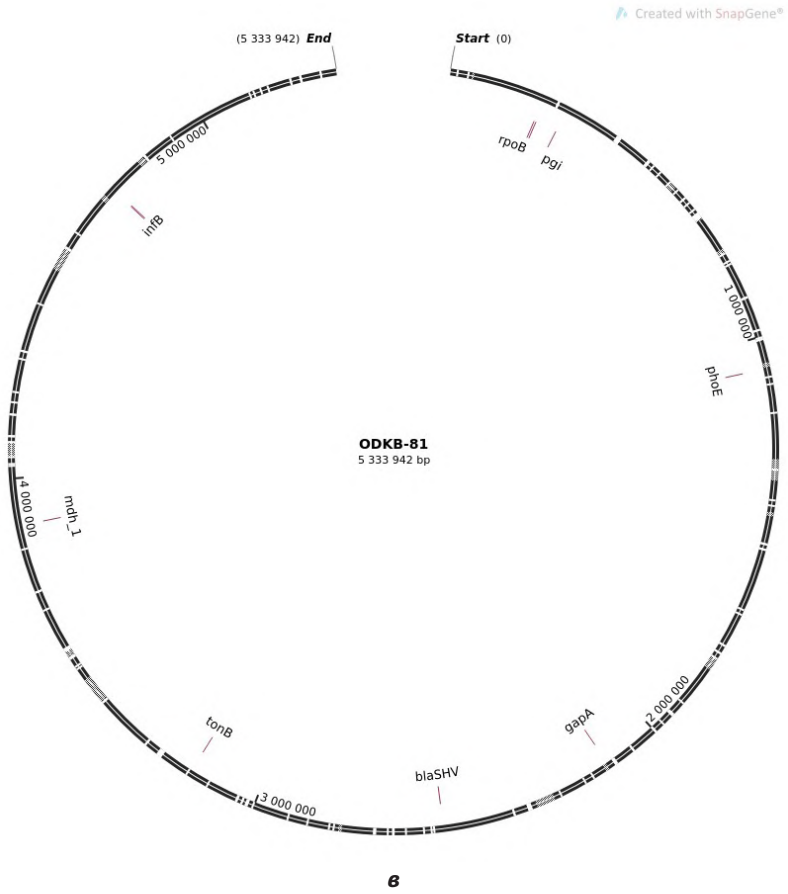

***FIG. 1.* (*continued*)**

Chromosome maps of K. pneumoniae ODKB-7 (а), K. pneumoniae ODKB-16 (б), K. pneumoniae ODKB-81 (в) strains mapped to the K. pneumoniae subsp. pneumoniae HS11286 reference genome. Genes identified by MLST (gapA, infB, mdh, pgi, phoE, rpoB, and tonB) as well as MLST analysis of virulence determinants (AbST, CbST, YbST, SmST, RmST) and antibiotic resistance are indicated in the footnotes

TABLE 4

GENETIC PROFILE OF ISOLATES OF THE *K. PNEUMONIAE* SPECIES COMPLEX

Isolate labeling	MLST	scgMLST629	scgMLST629_S Profile					
			LIN code	Phylogroup	Subline	Clonal group	K-type	KL-type
K. pneumoniae ODKB-16	ST-65	scgST-11107	0_0_391_0_0_0_3_1_0_0	Kp1	SL1471; SL322; SL65	CG1471; CG322; CG65	K2	KL2
K. pneumoniae ODKB-07	ST-219	scgST-6401	0_0_80_8_0_0_0_7_0_0	Kp1	SL107; SL3157	CG219; CG3157	no	KL125KL114
K. pneumoniae ODKB-81	ST-86	scgST-2800	0_0_395_0_13_1_0_0_0_0	Kp1	SL1471; SL322; SL86	CG1471; CG322; CG86	K2	KL2KL30

TABLE 5

VIRULENCE PROFILE OF *K. PNEUMONIAE* ODKB-16 ISOLATE

AbST/Aerobactin/ *iucABCD, iutA**		CbST/Colibactin/ *clbABCDEFGHILMNOPQ*		YbST/ Yersiniabactin/ *ybtSXQPAUTE, irp2, irp1, fyuA*	SmST/Salmochelin/ *iroBCDN*		RmST/RmpADCproteins/ *rmpACD*	
Genotype	**iuc lineage**	**Genotype**	**clb lineage**	**ICE lineage**	**Genotype**	**iro lineage**	**Genotype**	**rmp lineage**
ST-1	iuc 1	ST-13	clb 3	Ybt-17; ICEKp10	ST-10	iro 1	ST-38	rmp 1; KpVP-1

Note. * – gene cluster name/target product/list of genes from the gene cluster.

has been previously revealed to be closely associated with *K. pneumoniae* infection [29, 30]. All loci of this operon were only determined in the highly virulent MDR isolate of *K. pneumoniae* ODKB-16 (Fig. 16).

Resistome analysis of the studied strains using the Pasteur Institute's service for strains of the *K. pneumoniae* [27] revealed that the genome of three strains of *K. pneumoniae* contained gene clusters encoding resistance to aminoglycosides, beta-lactamases, quinolones, and had genotype 2b, determined by 23 loci encoding resistance to beta-lactam antibiotics. Additionally, *catA* and *sulA* genes encoding resistance to chloramphenicol and sulphonamides, respectively, were determined in the genomes of all strains. The *fosA* gene encoding resistance to fosfomycin was revealed only in the chromosome of *K. pneumoniae* isolates ODKB-16 and ODKB-81. Efflux pumps are considered to be one mechanism of multiple AMA resistance formation. Determinants of efflux pumps and their regulation *oqxAB, acrAB-tolC, acrZ, cusA, marAR, soxSR, rob, ramAR* (RND (Resistance-Nodulation Division) family), *mdtM, bcr* (MFS (Major Facilitator Superfamily) family), and *macAB* (ABC (ATP Binding Cassette) family) were determined in the genomes of all isolates.

DISCUSSION

In our previous studies, opportunistic bacteria of *K. pneumoniae* species were classified as an etiological factor in the development of nosocomial generalised purulent-septic infections [12], and their ability to biofilm formation and resistance to disinfectants and AMA was observed [13-15]. Three isolates of *K. pneumoniae* with resistance to different groups of AMA were studied to determine the clinical significance and potential of the *K. pneumoniae* species complex. It should be outlined that *K. pneumoniae* strains ODKB-07 and ODKB-81 were isolated from biotopes that account for the highest frequency of isolation of opportunistic microorganisms of the *K. pneumoniae* species complex [12] – these are both blood and sputum, respectively.

The phylogenetic analysis of the *K. pneumoniae* species complex conducted by M. Hennart et al. [3] allowed to isolate seven major phylogroups Kp1-Kp7, with the most represented phylogroup Kp1 – the group of *K. pneumoniae* sensu stricto. Various studies have revealed a great diversity of sublineages (SL) and clonal groups (CG) in its phylogenetic structure, reflecting the active interest of clinical microbiologists in isolates with multidrug resistance or/and hypervirulence [1, 3, 10, 11]. Phylogenetic analyses of other *K. pneumoniae* phylogroups revealed divergent SLs, but they were not predominant; apparently clinically important sublines and clonal groups in these phylogroups have yet to be sequenced [3]. The authors also complemented the multilocus core genome analysis scheme (cgMLST, 634 loci) previously defined by S. Bialek-Davenet et al. [2]. The scgMLST629 scheme includes 629 loci and the profile combines LIN code, phylogroup (Kp), sublineage (SL), and clonal group (CG) [3].

The *K. pneumoniae* isolates ODKB-16, ODKB-07, and ODKB-81 that have been analysed in this study were assigned to genotypes ST-65, ST-219, and ST-86 based on MLST analysis. ScgMLST629 analysis revealed similarity to sublineages SL1471, SL322, and SL65 for *K. pneumoniae* ODKB-16, SL107, and SL3157 for *K. pneumoniae* ODKB-07, and SL1471, SL322, and SL86 for *K. pneumoniae* ODKB-81.

It should also be pointed out that there is a different frequency of virulence genes and AMA resistance genes being observed among the major SLs and CGs [3, 31]. Among the analyzed isolates, all loci were determined only in the *K. pneumoniae* ODKB-16 genome, according to the virulence profile proposed earlier [10, 11]. An isolate with a mean virulence score of 5 had a mean resistance score of 1 (1 = ESBL).

The emergence of highly virulent strains of *K. pneumoniae* with high resistance to antibiotics has recently forced researchers to actively study the mechanisms and factors responsible for the emergence and survival of such bacteria [29]. Considering the effect of AMA against bacteria as a cell response to an environmental stressor, the formation of resistance may be a consequence of different genetic determinants. Alternatively, cross-resistance or jointly resistance, for example, to AMA and disinfectants and/or AMA and heavy metals, may be associated with similar

genetic mechanisms for resistance phenotype formation [32, 33]. In recent experimental studies with model animals, the tellurium resistance operon, known as *ter*-operon, was revealed to be associated with pneumonia and bacteraemia caused by *K. pneumoniae* [29]. Comprehensive studies of the *ter*-operon in *K. pneumoniae* based on genomic and bioinformatic approaches revealed that the *ter*-operon was genetically independent of other plasmid-encoded virulence and antibiotic resistance loci [29]. In mouse model experiments, *ter*-operon, which is closely associated with infection, has been revealed to encode factors that resist stress induced by the local gut bugs during *K. pneumoniae* colonisation [29]. In a study modelling urinary tract infection, the role of TerC protein in resistance to ofloxacin, polymyxin B, and cetylpyridinium chloride was revealed [30], and together, the results of these studies suggest a role for *ter*-operon as a factor in persistence and stress tolerance [29, 30]. Among the *K. pneumoniae* strains analysed in this study, only in the genome of the highly virulent isolate with MDR ODKB-16 all loci of the *ter*-operon were characterized, indicating the presence of additional determinants of tolerance to environmental stressors in its genome.

CONCLUSION

Phylogroups, sublines, and clonal groups within isolates of the *K. pneumoniae* species complex can vary considerably in their ecology and pathogenicity, and their precise definition is important in both basic research and practical public health. The genomes of three clinical isolates of *K. pneumoniae* obtained from the patients' clinical material were characterised based on different multilocus sequence typing schemes. The virulence profile was determined, MLST typing of seven genes and core genome typing with 629 genes (scgMLST62) was performed, and all isolates were assigned to the Kp1 phylogroup, K2 K-type and 2b genotype. Additionally, the *ter* operon has been characterized as a stress tolerance factor. This comprehensive species-specific scheme for genomic taxonomy of clinical isolates may be used and should help unify global and regional patterns of emergence and microevolution of bacterial pathogens.

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Conflict of interest

The authors of this article declare no conflicts of interest.

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