Multidrug-Resistant *Klebsiella pneumoniae* Causing Severe Infections in the Neuro-ICU

Nadezhda Fursova 1,*, Evgenii I. Astashkin 1, Olga N. Ershova 2, Irina A. Aleksandrova 2, Ivan A. Savin 2, Tatiana S. Novikova 1, Galina N. Fedyukina 3, Angelina A. Kislichkina 4, Mikhail V. Fursov 5, Ekaterina S. Kuzina 3, Sergei F. Biketov 6 and Ivan A. Dyatlov 6

1 Department of Molecular Microbiology, State Research Center for Applied Microbiology and Biotechnology, Territory “Kvartal A”, 142279 Obolensk, Russia; info@obolensk.ru (I.A.); savinova@nsi.ru (O.N.E.); biketov@obolensk.org (S.F.B.)

2 Department of Immunobiochemistry of Pathogenic Microorganisms, State Research Center for Applied Microbiology and Biotechnology, Territory “Kvartal A”, 142279 Obolensk, Russia; galafed@mail.ru (G.N.F.);

3 Department of Culture Collection, State Research Center for Applied Microbiology and Biotechnology, Territory “Kvartal A”, 142279 Obolensk, Russia; angelinakislichkina@yandex.ru

4 Department of Culture Collection, State Research Center for Applied Microbiology and Biotechnology, Territory “Kvartal A”, 142279 Obolensk, Russia; mikhail.fursov88@gmail.com (M.V.F.);

5 Department of Training and Improvement of Specialists, State Research Center for Applied Microbiology and Biotechnology, Territory “Kvartal A”, 142279 Obolensk, Russia; bikekova@nsi.ru (I.A.A.); info@nsi.ru (I.A.S.)

6 Department of Administration, State Research Center for Applied Microbiology and Biotechnology, Territory “Kvartal A”, 142279 Obolensk, Russia; dyatlov@obolensk.org

*Correspondence: n-fursova@yandex.ru*

**Abstract:** The purpose of this study was the identification of genetic lineages and antimicrobial resistance (AMR) and virulence genes in *Klebsiella pneumoniae* isolates associated with severe infections in the neuro-ICU. Susceptibility to antimicrobials was determined using the Vitek-2 instrument. AMR and virulence genes, sequence types (STs), and capsular types were identified by PCR. Whole-genome sequencing was conducted on the Illumina MiSeq platform. It was shown that *K. pneumoniae* isolates of ST14 K2, ST23 K57, ST39 K2, ST76 K2, ST86 K76, ST18 K57, ST21 K125/114, ST268 K20, and ST2674 K47 caused severe systemic infections, including ST14 K2, ST39 K2, and ST268 K20 that were associated with fatal incomes. Moreover, eight isolates of ST395 K2 and ST307 K102/149/155 were associated with manifestations of vasculitis and microcirculation disorders. Another 12 *K. pneumoniae* isolates of ST395 K2.KL39, ST307 K102/149/155 and ST147 K14/64 were collected from patients without severe systemic infections. Major isolates (n = 38) were XDR and MDR. Beta-lactamase genes were identified: blasHV (n = 41), blasCTX-M (n = 28), blasTEM (n = 21), blasOXA-48 (n = 21), blasNDM (n = 1), and blasKPC (n = 1). The prevalent virulence genes were wabG (n = 41), fimH (n = 41), allS (n = 41), and uge (n = 34), and rarer, detected only in the genomes of the isolates causing severe systemic infections—rmpA (n = 8), kfu (n = 6), iroN (n = 5), and iroD (n = 5) indicating high potential of the isolates for hypervirulence.

**Keywords:** *Klebsiella pneumoniae*; healthcare-associated infections; microbial drug resistance; sequence type; capsular type; whole genome sequencing

**1. Introduction**

Healthcare-associated infections (HAI) have posed a huge medical burden to public health worldwide. *Klebsiella pneumoniae* is one of the clinically significant nosocomial pathogens causing broad spectra of diseases and showing increasingly frequent acquisition of resistance to antibiotics including in intensive care units (ICU) [1]. Today, according to the BIGSDB Institute Pasteur database (https://bigsdb.pasteur.fr/ access date 8 August 2021), 5797 *K. pneumoniae* sequence types and 711 capsular types have been discovered.
K. pneumoniae causes different infections in ICU patients associated with high mortality rates, including bloodstream infection, pulmonary infection, and healthcare-associated ventriculitis, meningoencephalitis, and urinary tract infection [2,3]. It was reported from China, Spain, and Taiwan that K. pneumoniae was associated with leukocytoclastic vasculitis and microcirculation disorders, but there is no data on the relationship of such a clinical manifestation with any K. pneumoniae genetic lineages [4–6].

Current data suggest that genetic determinants associated with antimicrobial resistance (AMR) and virulence are specific for distinct genetic lineages of K. pneumoniae [7]. Classical K. pneumoniae (cKP) are the common healthcare-associated pathogens causing nosocomial infections in immunocompromised patients and characterized as a great possibility to accumulate resistance mechanisms to antimicrobials—multidrug-resistant (MDR), extensive drug-resistant (XDR), and pan drug-resistant (PDR) strains have been described [8]. Carbapenems are the last antibiotics available to control K. pneumoniae infection. However, to date, many mechanisms involved in carbapenem resistance in K. pneumoniae have already been described, including carbapenemase production. Strains producing carbapenemases of functional class A (KPC), class B (NDM), and class D (OXA-48), and co-producing more than one type of carbapenemase are increasingly reported [9].

For hypervirulent K. pneumoniae (hvKP), many critical virulence factors have been discovered, among them rmpA (regulator of mucoid phenotype), aerobactin (an iron siderophore), kfu (an iron uptake system), alls (associated with allantoin metabolism), and K1/K2 capsules [10]. The global spread of ‘convergent’ K. pneumoniae strains that combines the pathogenic potentials of cKP and hvKP has occurred since 2010. Three types of the mechanism for the emergence of ‘convergent’ clones have been identified: (i) cKP acquiring a hypervirulence plasmid; (ii) hvKP acquiring a carbapenem-resistant plasmid; and (iii) K. pneumoniae acquiring both a carbapenem resistance and hypervirulence hybrid plasmid. These strains are at high risk to disseminate and have the potential to cause severe infections [9].

This study aimed to examine the phenotypes, genotypes, and genetic relatedness of K. pneumoniae isolates collected from the patients of neuro-ICU and evaluated the sequence types associated with severe infections between 2017 and 2019.

2. Results
2.1. Patients and Bacterial Strains

During the period from Oct. 2017 to Jan. 2019, the following incidence of infections was detected in neurosurgery ICU: 8.4 infections of the central nervous system per 100 patients, 2.7/100 of bloodstream infections, 26.3/100 of ventilator-associated pneumonia, and 23.6/100 of urinary tract infections. K. pneumoniae accounted for 33, 31, 23, and 25% among the agents of named infections, correspondingly [3]. The subject of this study was 41 resistant-to-antimicrobials K. pneumoniae isolates collected from 20 patients with severe postoperative infections (Table 1).

Table 1. Clinical data of the patients: underlying disease, infections, antimicrobial treatment, and outcomes.

| Patient | Neurosurgery Disease | Infection | Antimicrobial Treatment | Outcome          |
|---------|----------------------|-----------|------------------------|------------------|
| A       | Hemorrhagic stroke.  | Meningitis, abscess, and hemorrhage in the right frontal lobe of the brain, encephalitis, ventriculitis, epidural empyema, erythematous maculae and vesicles | Meropenem       | Discharged       |
|         |                      |           | Tigecycline            |                  |
| D       | Multiple cerebral aneurysms, subarachnoid hemorrhage into the ventricular system. | Systemic inflammatory response, sepsis, septic shock, intracerebral hematoma | Cefoperazone-sulbactam | Died             |
|         |                      |           | Meropenem              |                  |
|         |                      |           | Colistin (inhalation)  |                  |
| Patient | Neurosurgery Disease | Infection | Antimicrobial Treatment | Outcome |
|---------|----------------------|-----------|------------------------|---------|
| F       | Rupture of aneurysm of the anterior cerebral artery, subarachnoid hemorrhage, ventriculoperitoneal shunt. | Meningitis, long-term (4 weeks) eradication of Klebsiella from cerebrospinal fluid, erythematous macules and vesicles | Meropenem Colistin (intrathecal) | Discharged |
| I       | Multiple cerebral aneurysms, subarachnoid hemorrhage into the ventricular system, endovascular occlusion of the basilar artery aneurysm, basilar artery thrombosis. | Multiple focal hemorrhages of supra- and subtentorial localization, erythematous macules and vesicles | Meropenem Tigecycline Amikacin | Discharged |
| J       | Odontoid process fracture of the C2 vertebra, dorsal fixation of the C1–C2 vertebrae. | Severe inflammatory reaction, increased markers of systemic inflammation, 50 mL of pus with blood was removed from the surgical wound. | Tigecycline, Meropenem, Ceftazidime-avibactam, Bacteriophage | Discharged |
| K       | Complex defect of the base of the skull, spontaneous nasal liquorthea, ventriculoperitoneal shunt. | Meningitis, thrombus in the pulmonary artery trunk, encephalitis, ventriculitis. | Meropenem Ciprofloxacin Amikacin (intrathecal) | Discharged |
| L       | Mature teratoma of the chiasmal-sellar region, antitumor chemotherapy, removal of the teratoma | Meningitis, encephalitis, sepsis, severe septic reaction. | Meropenem | Discharged |
| M       | Open penetrating traumatic brain injury, cerebral contusion, cerebral edema, subarachnoid hemorrhage. | Pneumonia with middle level markers of systemic inflammatory response. | Meropenem, Tigecycline Amikacin Colistin (inhalation) | Discharged |
| N       | Large partially thrombosed cerebellar artery aneurysm, subarachnoid parenchymal hemorrhage, aneurysm clipping surgery. | Sepsis, septic reaction with markers of inflammation | Doripenem Amikacin Sulperason | Discharged |
| P       | Gunshot wound to the skull and brain, Intraventricular hemorrhage, subdural hematoma of the frontal-parietal-temporal region | Klebsiella meningoencephalitis had a long persistent course, subarachnoid hemorrhage; markers of systemic inflammation were increased. | Meropenem Colistin | Discharged |
| Q       | Closed traumatic brain injury, brain contusion with multiple hemorrhagic foci, subarachnoid hemorrhage, and occipital bone fracture. | Severe pneumonia, urinary infection, systemic inflammatory response, a rapid increase in inflammatory markers. | Tigecycline Meropenem | Died |
| R       | Anaplastic ependymoma, tumor removal. | Fever, increased leukocytosis, markers of systemic inflammatory response, sepsis, meningitis | Meropenem, Ciprofloxacin | Discharged |
| S       | Aneurysm of the right internal carotid artery, subarachnoid hemorrhage, aneurysm climax, ventricular drainage. | Meningoencephalitis, infection of the ventriculoperitoneal shunt, purulent masses in the lateral ventricles of the brain, pansinusitis. | Doripenem Colistin (inhalation) Colistin (intrathecal) | Died |
| B       | Rupture of middle cerebral artery aneurysms. | Urinary infection with increased markers of inflammation, fever. | Meropenem Tigecycline | Discharged |
| C       | Ruptured aneurysm of the internal carotid artery. | Pansinusitis, no markers of inflammation. | Cefoperazone-sulbactam Meropenem | Discharged |

**Table 1. Cont.**

Group B—patients without severe manifestations of systemic infections
Table 1. Cont.

| Patient | Neurosurgery Disease                                                                 | Infection                                                                                     | Antimicrobial Treatment                      | Outcome          |
|---------|--------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|----------------------------------------------|------------------|
| E       | Pilocytic astrocytoma, tumor biopsy, ventriculoperitoneal shunt.                     | Urinary infection without systemic markers of inflammation.                                    | Amoxicillin-clavulanic acid                  | Discharged       |
| G       | Multiple metastases of kidney cancer in the ventricles of the brain, tumor removal.  | Pneumonia with low markers of systemic inflammatory response. Died of an underlying disease.   | Cefoperazone-sulbactam Meropenem             | Died             |
| H       | Craniopharyngioma, tumor removal.                                                    | Pneumonia without systemic markers of inflammation.                                           | Meropenem                                    | Discharged       |
| O       | Closed traumatic brain injury, subdural hematoma, cerebral edema, subarachnoid hemorrhage. | There was no septic reaction; markers of inflammation did not increase.                       | Meropenem, Tigecycline Colistin (inhalation)  | Discharged       |
| T       | Arteriovenous malformation of cerebral vessels.                                     | Tracheobronchitis, bacteriuria, leukocyturia.                                                 | Sulfamethoxazole/Trimethoprim, Meropenem     | Discharged       |

Depending on clinical manifestations, isolates were combined into two groups. Group A included 29 isolates collected from 13 patients (D, J, Q, P, M, S, L, K, N, R, A, I, and F) with the pronounced systemic inflammatory response (SIRS). Symptoms had been reported for the patients: fever >38 °C, leukocytosis, increased markers of systemic inflammation, multiple hemorrhages, thrombosis, or high risk of thromboembolism. Clinical forms had been detected: meningitis after the neurosurgical intervention, pansinusitis, pneumonias, sepsis and septic shock, ventriculitis, and brain abscess. In this group, seven isolates (B-548/18, B-784/18, B-1154/18, B-1618/18, B-2625/18, B-14/19, and B-21/19) were associated with fatal incomes collected from the patients D, Q, and S died due to increased symptoms of inflammation, sepsis, septic shock, meningitis associated with K. pneumoniae infection. Other eight isolates (B-3002K/17, B-3060K/17, B-2016K/17, B-3299/17, B-1040/18-1, B-792/18, B-853/18-1, and B-1214/18-2) of Group A were associated with manifestations of vasculitis and microcirculation disorders (patients A, I, and F). Major isolates of Group A (17/29) were collected from the blood and cerebrospinal fluid, less (7/29)—from the endotracheal aspirate.

Group B consists of 12 strains isolated from the patients without increasing markers of systemic inflammation and septic reaction. Major isolates of Group B were from the endotracheal aspirate and urine (10/12) and the rest from the nervous system. One patient in this group died due to the underlying disease, videlicet multiple metastases of kidney cancer in the brain (Tables 1 and 2). It should be noted that isolates of Group A were obtained from 2–3 body sites of one patient: three isolates—from blood, urine, and cerebrospinal fluid of the patient D; three isolates—from endotracheal aspirate and cerebrospinal fluid of patient S; and three isolates—from blood and cerebrospinal fluid of the patient L (Table 2).

Table 2. Sources of isolation and antimicrobial resistance phenotypes of K. pneumoniae strains.

| Strain   | Date      | Source | Patient | Resistance Phenotype | Resistance Category |
|----------|-----------|--------|---------|----------------------|---------------------|
| B-548/18 | 02-Apr-2018 | trachea | D #     | BL, ACE, TET, QNL, CM, AMI, SUL, NIT | XDR                 |
| B-784/18 | 03-May-2018 | urine  | D #     | BL, ACE, TET, QNL, CM, AMI, SUL, NIT | XDR                 |
| B-1154/18| 03-May-2018 | blood  | D #     | BL, ACE, TET, QNL, CM, AMI, SUL, NIT | XDR                 |
| B-1396/18-2 | 25-May-2018 | blood  | J       | BL, ACE, TET, QNL, CM, AMI, SUL, NIT | XDR                 |
| B-1618/18 | 27-Aug-2018 | urine  | Q #     | BL, ACE, TET, QNL, CM, AMI, SUL, NIT | XDR                 |
## Table 2. Cont.

| Strain        | Date       | Source | Patient | Resistance Phenotype | Resistance Category |
|---------------|------------|--------|---------|----------------------|---------------------|
| B-2035K/18-2  | 23-Jul-2018| CSF    | P       | BL<sub>ACE</sub>, TET, QNL, CM, AMI, SUL, NIT | XDR                |
| B-2062/18     | 25-Jul-2018| CSF    | P       | BL<sub>ACE</sub>, TET, QNL, CM, AMI, SUL, NIT | XDR                |
| B-2086/18     | 27-Jul-2018| CSF    | P       | BL<sub>ACE</sub>, TET, QNL, CM, AMI, SUL, NIT | XDR                |
| B-968/18-1    | 30-May-2018| trachea| M       | BL<sub>ACE</sub>, TET, QNL, CM, SUL, NIT     | XDR                |
| B-1120K/18    | 20-Jun-2018| trachea| M       | BL<sub>ACE</sub>, TET, QNL, CM, SUL, NIT     | XDR                |
| B-2625/18     | 12-Dec-2018| trachea| S<sup>#</sup> | BL<sub>ACEI</sub>, TET, QNL, CM, AMI, NIT | XDR                |
| B-14/19       | 09-Jan-2019| CSF    | S<sup>#</sup> | BL<sub>ACEI</sub>, TET, QNL, CM, AMI, NIT | XDR                |
| B-21/19       | 19-Jan-2019| trachea| S<sup>#</sup> | BL<sub>ACEI</sub>, TET, QNL, CM, AMI, NIT | XDR                |
| B-1398/18-1   | 25-May-2018| blood  | L       | BL<sub>AC</sub>, TET, CM, SUL              | MDR                |
| B-1406/18-1   | 25-May-2018| CSF    | L       | BL<sub>AC</sub>, TET, CM, SUL              | MDR                |
| B-1412/18-1   | 25-May-2018| blood  | L       | BL<sub>AC</sub>, TET, CM, SUL              | MDR                |
| B-1230/18-1   | 10-May-2018| CSF    | K       | BL<sub>AE</sub>, CM, NIT                  | MDR                |
| B-849/18-2    | 11-May-2018| trachea| K       | BL<sub>AE</sub>, CM, NIT                  | MDR                |
| B-1207/18     | 03-Jul-2018| blood  | N       | BL<sub>AC</sub>                             | R                  |
| B-1636/18     | 30-Aug-2018| urine  | R       | BL<sub>AE</sub>                             | R                  |
| B-2523/18     | 30-Aug-2018| blood  | R       | BL<sub>AE</sub>                             | R                  |
| B-3002K/17*   | 30-Oct-2017| CSF    | A       | BL<sub>ACE</sub>, TET, QNL, CM, AMI, SUL, NIT | XDR                |
| B-3060K/17*   | 03-Nov-2017| CSF    | A       | BL<sub>ACE</sub>, TET, QNL, CM, AMI, SUL, NIT | XDR                |
| B-2016K/17*   | 14-Nov-2017| BA     | A       | BL<sub>ACE</sub>, TET, QNL, CM, AMI, SUL, NIT | XDR                |
| B-3299/17*    | 24-Nov-2017| CSF    | A       | BL<sub>ACE</sub>, TET, QNL, CM, AMI, SUL, NIT | XDR                |
| B-1040/18-1*  | 09-Jun-2018| trachea| I       | BL<sub>AE</sub>, QNL, CM, AMI, SUL, NIT     | XDR                |
| B-792/18*     | 03-May-2018| trachea| I       | BL<sub>AE</sub>, QNL, CM, NIT              | MDR                |
| B-853/18-1*   | 14-May-2018| trachea| I       | BL<sub>ACEI</sub>, QNL, NIT                | MDR                |
| B-1214/18-2*  | 08-May-2018| CSF    | F       | BL<sub>AC</sub>, TET, QNL, SUL             | MDR                |

**Group B—strains not associated with severe manifestations of systemic infections**

| Strain        | Date       | Source | Patient | Resistance Phenotype | Resistance Category |
|---------------|------------|--------|---------|----------------------|---------------------|
| B-789/18-1    | 03-May-2018| urine  | E       | BL<sub>ACE</sub>, TET, QNL, AMI, SUL, NIT | XDR                |
| B-851/18-1    | 14-May-2018| urine  | E       | BL<sub>ACE</sub>, TET, QNL, AMI, SUL, NIT | XDR                |
| B-790/18-1    | 03-May-2018| trachea| G<sup>#</sup> | BL<sub>ACEI</sub>, QNL, CM, AMI, SUL, NIT | XDR                |
| B-823/18-1    | 07-May-2018| trachea| G<sup>#</sup> | BL<sub>ACEI</sub>, QNL, CM, AMI, SUL, NIT | XDR                |
| B-702/18      | 20-Apr-2018| IS     | C       | BL<sub>ACEI</sub>, TET, QNL, AMI, SUL, NIT | XDR                |
| B-771/18      | 28-Apr-2018| urine  | B       | BL<sub>AEI</sub>, QNL, CM, AMI, SUL, NIT  | XDR                |
| B-102/19      | 21-Jan-2019| urine  | T       | BL<sub>ACEI</sub>, TET, QNL, CM, AMI, SUL, NIT | XDR                |
| B-543/18      | 02-Apr-2018| CSF    | H       | BL<sub>ACEI</sub>, TET, QNL, CM, AMI, SUL, NIT | XDR                |
| B-587/18      | 09-Apr-2018| trachea| H       | BL<sub>ACEI</sub>, QNL, CM, AMI, SUL, NIT  | XDR                |
| B-775/18-1    | 03-May-2018| trachea| H       | BL<sub>AEI</sub>, QNL, CM, AMI, SUL, NIT  | XDR                |
| B-691/18-4    | 19-Apr-2018| trachea| H       | BL<sub>ACE</sub>, QNL, CM, NIT             | MDR                |
| B-1363/18-1   | 24-Jul-2018| trachea| O       | BL<sub>ACEI</sub>, QNL, AMI, NIT           | MDR                |

Abbreviations: *, strains associated with systemic manifestations of vasculitis and microcirculation disorders; CSF, cerebrospinal fluid; BA, brain abscess; IS, intracranial sinus; BL<sub>ACEI</sub>, beta-lactams (ampicillin, cephalosporins, ertapenem, imipenem); TET, tetracyclines; QNL, fluoroquinolones; CM, chloramphenicol; AMI, aminoglycosides; SUL, sulfonamides; NIT, nitrofurans; R, resistant; MDR, multidrug resistant; XDR, extremely drug resistant; #, patient died.
2.2. Susceptibility to Antimicrobials

According to Magiorakos et al. [11] criteria, 28 isolates were attributed to the XDR category (resistant to 6–7 functional groups of antimicrobials), 10 isolates to the MDR category (resistant to 3–4 functional groups), and 3 isolates to the R category (resistant to ampicillin only). XDR isolates were attributed to Groups A (n = 18) and B (n = 10); MDR isolates—to the Groups A (n = 8) and B (n = 2); R isolates—only to Group A (n = 3). All isolates were resistant to beta-lactams, 34 isolates—to nitrofurans, 33 isolates—to fluoroquinolones, 29 isolates—to chloramphenicol, 29 isolates—to sulfonamides, 27 isolates—to aminoglycosides, 26 isolates—to tetracyclines. Major isolates were susceptible to amikacin (n = 33) and imipenem (n = 27), all isolates were susceptible to colistin (Figure 1, Table S1).

Figure 1. Rate of K. pneumoniae isolates resistant to antimicrobials: AMP, ampicillin; SAM, ampicillin-sulbactam; CXM, cefuroxime; FOX, cefoxitin; CAZ, ceftazidime; SCF, cefoperazone-sulbactam; FEP, cefepime; ETP, ertapenem; IPM, imipenem; TET, tetracycline; TGC, tigecycline; CIP, ciprofloxacin; CHL, chloramphenicol; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; SXT, trimethoprim-sulfamethoxazole; NIT, nitrofurantoin; CST, colistin.

2.3. Beta-Lactamase Genes and Integrons

Total 113 beta-lactamase genes were identified in K. pneumoniae isolates, including blaSHV (n = 41), blaCTX-M (n = 28), blaTEM (n = 21), blaOXA-48 (n = 21), blaNDM (n = 1), and blaKPC (n = 1). The blaVIM and blaIMP genes were not detected. Number of beta-lactamase genes per strain varied from one to five. Single blaSHV gene was detected in 4 strains; two genes (blaSHV + blaCTX-M) or (blaSHV + blaOXA-48)—in 4 and 9 strains, respectively; three genes (blaSHV + blaCTX-M + blaOXA-48) or (blaSHV + blaCTX-M + blaTEM)—in 3 and 11 strains, respectively; four genes (blaSHV + blaCTX-M + blaTEM + blaNDM) or (blaSHV + blaCTX-M + blaTEM + blaOXA-48)—in 1 and 8 strains, respectively; five genes (blaSHV + blaCTX-M + blaTEM + blaOXA-48 + blaKPC) were identified in one strain. Moreover, 10 strains carried class 1 integrons, and one strain carried two integrons, class 1 and class 2 simultaneously (Table 3).

Table 3. Molecular-genetic characteristics of K. pneumoniae strains.
Table 3. Cont.

| Strain     | Beta-Lactamase Genes | Int       | Virulence Genes                     | ST  | Capsular Type |
|------------|----------------------|-----------|-------------------------------------|-----|---------------|
| B-968/18-1 | blaSHV, blaCTX-M, blaOXA-48 |           | rmpA, wabG, fimH, allS              | 23  | K57           |
| B-1120K/18 | blaSHV, blaCTX-M, bltemps, blaOXA-48 |           | rmpA, wabG, fimH, allS              | 23  | K57           |
| B-2625/18  | blaSHV, blaCTX-M, bltemps, blaOXA-48 |           | uge, wabG, fimH, allS               | 39  | K23           |
| B-14/19    | blaSHV, blaCTX-M, bltemps, blaOXA-48, blkPC |           | uge, wabG, fimH, allS               | 39  | K23           |
| B-21/19    | blaSHV, blaCTX-M, bltemps, blaOXA-48 |           | uge, wabG, fimH, allS               | 39  | K23           |
| B-1398/18-1| blaSHV, blaCTX-M          |           | uge, wabG, kfu, fimH, allS          | 219 | KL125/114     |
| B-1406/18-1| blaSHV, blaCTX-M          | int1      | uge, wabG, kfu, fimH, allS          | 219 | KL125/114     |
| B-1412/18-1| blaSHV, blaCTX-M          |           | uge, wabG, kfu, fimH, allS          | 219 | KL125/114     |
| B-3002K/17 | blAWSH                 |           | rmpA, kfu, wabG, fimH, allS          | 218 | K57           |
| B-849/18-2 | blAWSH                 |           | rmpA, kfu, wabG, fimH, allS          | 218 | K57           |
| B-1230/18-1| blAWSH, blaCTX-M         |           | rmpA, uge, wabG, fimH, allS          | 76  | K23           |
| B-1636/18  | blAWSH                 |           | rmpA, irON, irOD, uge, wabG, fimH, allS | 86  | K2            |
| B-2523/18  | blAWSH                 |           | rmpA, irON, irOD, uge, wabG, fimH, allS | 86  | K2            |
| B-3002K/17 *| blAWSH, blaCTX-M, bltemps |           | uge, wabG, fimH, allS               | 395 | K2            |
| B-3060K/17 *| blAWSH, blaCTX-M, bltemps |           | uge, wabG, fimH, allS               | 395 | K2            |
| B-2016K/17 *| blAWSH, blaCTX-M, bltemps |           | uge, wabG, fimH, allS               | 395 | K2            |
| B-3299/17 *| blAWSH, blaCTX-M, bltemps |           | uge, wabG, fimH, allS               | 395 | K2            |
| B-1040/18-1 *| blAWSH, blaOXA-48       |           | uge, wabG, fimH, allS               | 395 | K2            |
| B-792/18 *  | blAWSH, blaOXA-48       | int1      | uge, wabG, fimH, allS               | 395 | K2            |
| B-853/18-1 *| blAWSH, blaOXA-48       |           | uge, wabG, fimH, allS               | 395 | K2            |
| B-1214/18-2 *| blAWSH, blaCTX-M, bltemps |           | uge, wabG, fimH, allS               | 307 | KL102/149/155 |

Group B—strains not associated with severe manifestations of systemic infections

| Strain     | Beta-Lactamase Genes | Int       | Virulence Genes                     | ST  | Capsular Type |
|------------|----------------------|-----------|-------------------------------------|-----|---------------|
| B-789/18-1 | blAWSH, blaCTX-M, bltemps |           | uge, wabG, fimH, allS               | 307 | KL102/149/155 |
| B-851/18-1 | blAWSH, blaCTX-M, bltemps |           | int1                                | 307 | KL102/149/155 |
| B-790/18-1 | blAWSH, blaOXA-48     |           | int1                                | 395 | K2            |
| B-823/18-1 | blAWSH, blaOXA-48     |           | uge, wabG, fimH, allS               | 395 | K2            |
| B-702/18   | blAWSH, blaCTX-M, bltemps, blaOXA-48 |       | uge, wabG, fimH, allS               | 395 | K2            |
| B-771/18   | blAWSH, blaCTX-M, blaOXA-48     | int1      | uge, wabG, fimH, allS               | 395 | K2            |
| B-102/19   | blAWSH, blaOXA-48     |           | int1                                | 395 | KL39          |
| B-543/18   | blAWSH, blaOXA-48     | int1      | uge, wabG, fimH, allS               | 395 | K2            |
| B-587/18   | blAWSH, blaCTX-M, blaOXA-48 |           | uge, wabG, fimH, allS               | 395 | K2            |
| B-775/18-1 | blAWSH, blaOXA-48     |           | uge, wabG, fimH, allS               | 395 | K2            |
| B-691/18-4 | blAWSH, blaOXA-48     |           | uge, wabG, fimH, allS               | 395 | K2            |
| B-1363/18-1| blAWSH, blaCTX-M, bltemps, blaNDM |     | uge, wabG, fimH, allS               | 147 | K14/64        |

Abbreviations: *, strains associated with systemic manifestations of vasculitis and microcirculation disorders; blAWSH, blaCTX-M, bltemps, blaOXA-48, blkPC, blaNDM, beta-lactamase genes; int1, int2, integrase class 1 and 2 genes; rmpA, hypermucoid phenotype regulator gene; irON, catecholate siderophore receptor gene; irOD, aerobactin esterase gene; kfu, ferric absorption system gene; uge, uridine diphosphate galacturonate-4-epimerase gene; wabG, glucosyltransferase gene; fimH, fimbria type I gene; allS, allantoin regulon; ST, sequence type.

2.4. Virulence Genes

The prevalent virulence genes detected in 41 K. pneumoniae clinical isolates were wabG (n = 41), fimH (n = 41), allS (n = 41), and uge (n = 34). Other virulence genes were rare and attributed only to the Group A: rmpA (n = 8), kfu (n = 6), irON (n = 5), and irOD (n = 5). Eight
combinations of 3 to 8 virulence genes were identified. Combination of three genes (\textit{wabG + fimH + allS}) was detected in three isolates; combinations of four genes (\textit{uge + wabG + fimH + allS}) and (\textit{rmpA + wabG + fimH + allS})—in 24 and 2 isolates, respectively; combinations of five genes (\textit{uge + wabG + kfu + fimH + allS}) and (\textit{rmpA + uge + wabG + fimH + allS})—in 6 and 1 isolates, respectively; combinations of six genes (\textit{rmpA + iroN + iroD + wabG + fimH + allS})—in 2 isolates; combinations of seven genes (\textit{rmpA + iroN + iroD + uge + wabG + fimH + allS})—in 3 isolates (Table 3).

2.5. Sequence Types and Capsular Types

It was found that \textit{K. pneumoniae} clinical isolates belonged to 12 sequence types: ST14 (\(n = 3\)), ST23 (\(n = 5\)), ST39 (\(n = 3\)), ST76 (\(n = 1\)), ST86 (\(n = 2\)), ST147 (\(n = 1\)), ST218 (\(n = 2\)), ST219 (\(n = 3\)), ST268 (\(n = 1\)), ST307 (\(n = 3\)), ST395 (\(n = 16\)) and ST2674 (\(n = 1\)). Nine sequence types (ST14, ST23, ST39, ST76, ST86, ST218, ST219, ST268, and ST2674) were identified only in Group A. One sequence type (ST147) was obtained only in Group B. Two sequence types (ST395 and ST307) were identified both in Group A (8 isolates associated with systemic manifestations of vasculitis and microcirculation disorders) and in Group B.

All studied \textit{K. pneumoniae} isolates were attributed to nine capsular types: K2 (\(n = 20\)), K20 (\(n = 1\)), K23 (\(n = 4\)), KL39 (\(n = 1\)), K47 (\(n = 1\)), K102/149/155 (\(n = 1\)), and K125/114 (\(n = 3\)). It was interesting in that \textit{K. pneumoniae} isolates of capsular type K2 belonged to three sequence types (ST14, ST86, and ST395), isolates of capsular type K23—to two sequence types (ST39 and ST76), and isolates of capsular type K57—to two sequence types (ST23 and ST218). The remaining capsular types were associated with only one sequence type: K20—ST268, KL39—ST395, K47—ST2674, K14/64—ST147, K102/149/155—ST307, and K125/114—ST219 (Table 3).

2.6. Phylogenetic Analysis

The phylogenetic tree was constructed on the base of combined gene sequences of MLST profiles; two clusters were revealed. Cluster I consisted of one ST147 referring to one isolate not associated with severe manifestations of systemic infections (Group B). Cluster II included two sub-clusters: Ila consisting of one ST86 associated with a pronounced systemic inflammatory response (Group A); IIb consisting of 10 genetic lineages including 8 sequence types (ST14, ST23, ST39, ST76, ST218, ST219, ST268, and ST2674) referred to the strains of Group A, and 2 sequence types (ST307 and ST396) including isolates both Group A and Group B (Figure 2).

![Figure 2](image-url)
2.7. Whole-Genome Sequencing

Whole-genome sequencing was done for nine isolates including eight isolates of Group A belonging to sequence types/capsular types ST2674/K47, ST23/K57, ST39/K23, ST219/K125, ST218/K57, ST76/K23, ST86/K2, and ST307/K102 and one isolate of Group B belonging to ST395/K39. From 86 to 164 contigs for each genome were obtained, the genome size ranged from 5.42 to 5.86 Mb, with the GC content ranging from 55.1 to 57.9%, and the number of genes from 5121 to 5845. All genomes carried 1–6 beta-lactamase genes including \( \text{bla}_{\text{SHV}} \), \( \text{bla}_{\text{TEM}} \), \( \text{bla}_{\text{CTX-M}} \), \( \text{bla}_{\text{OXA}} \), and \( \text{bla}_{\text{KPC}} \) types. Six alleles were identified of the \( \text{bla}_{\text{SHV}} \) gene (26, 28, 33, 40, 59, and 182), two alleles of \( \text{bla}_{\text{OXA}} \) genes (1 and 48), and other beta-lactamase genes were presented as only one allele (\( \text{bla}_{\text{TEM-1B}} \), \( \text{bla}_{\text{CTX-M-15}} \), and \( \text{bla}_{\text{KPC-2}} \)). The genetic environments of carbapenemase genes \( \text{bla}_{\text{KPC-2}} \) and \( \text{bla}_{\text{CTX-M-48}} \) and ceftazidimease gene \( \text{bla}_{\text{CTX-M-15}} \) were similar to previous descriptions of these genes. The gene coding of a putative transposition helper protein ISKpn7 was identified upstream \( \text{bla}_{\text{KPC-2}} \) gene, and ISKpn6 transposase gene downstream, that was similar to the genetic structure in the plasmid pBK32179 (IX430448). The environment for \( \text{bla}_{\text{OXA-48}} \) genes contained upstream, the transcriptional regulator LysR gene, and the transposase tir gene downstream, as in the plasmid pOXA-48 (JN626286). The surrounding genetic conditions for \( \text{bla}_{\text{CTX-M-15}} \) genes in clinical isolates were likewise similar to the plasmid pKP848CTX (NC_024992): ISecP1 family transposase upstream and gene coding WbuC family cupin fold metalloprotein downstream. Aminoglycoside modifying enzyme genes were identified in all but one isolate: 1–3 genes per isolate (\( \text{aac}, \text{aad}, \text{ant}, \text{aph}, \text{arm}, \text{and rmt} \)). All genomes carried the \( \text{fosA} \) gene conferring resistance to fosfomycin. Phenicol resistance genes (4 alleles of \( \text{cat} \) gene) were detected in seven genomes. Six isolates carried 2–4 quinolone resistance genes \( \text{qnr} \). Two alleles of \( \text{soI} \) gene (sulfonamide resistance) were identified in six genomes, \( \text{tet} \) gene (tetracycline resistance) in 5 genomes, four alleles of \( \text{dfrA} \) gene (trimethoprim resistance) in 4 genomes, and \( \text{mph} \) gene (macrolide resistance) in only one genome. The genes encoding efflux pumps were revealed in all isolates, seven of them carried 10 genes, two isolates—9 genes. Moreover, all genomes carried 1–4 genes encoding heavy metal resistance (to copper, lead, silver, arsenic, and tellurium) (Table 4).

Table 4. Molecular-genetic characteristics of \( \text{K. pneumoniae} \) isolates based on Whole-Genome Sequencing.

| Isolate      | B1396/18-2 | B1120K/18 | B14/19 | B1406/18-1 | B1230/18-1 | B1207/18 | B2523/18 | B1214/18-2 | B102/19 |
|--------------|------------|------------|--------|------------|------------|----------|----------|------------|---------|
| Source       | blood      | blood      | blood  | blood      | blood      | blood    | blood    | blood      | blood   |
| Date         | 25-May-2018| 20-Jun-2018| 09-Jan-2019| 25-May-2018| 10-May-2018| 03-Jul-2018| 30-Aug-2018| 08-May-2018| 21-Jan-2019|
| BioSample ID | SAMN18679 | SAMN18679 | SAMN18679 | SAMN18679 | SAMN18679 | SAMN18679 | SAMN18679 | SAMN18679 | SAMN18679 |
| GenBank      | JAGRZJ00000| JAGRZJ00000| JAFFJK00000| JAGRZJ00000| JAFFJK00000| JAGRZJ00000| JAFFJK00000| JAGRZJ00000| JAFFJK00000|
| G+C-content, %| 56.76      | 57.07      | 56.76   | 57.26      | 57.29      | 56.9     | 57.39    | 57.38      | 57.24    |
| Reads        | 1,461,812  | 1,268,077  | 4,656,796| 1,031,631  | 904,468    | 920,438  | 659,438  | 932,557    | 939,118  |
| Contigs, n   | 168        | 103        | 164     | 94         | 86         | 117      | 125      | 93         | 154      |
| Genome, bp   | 5,777,126  | 5,581,990  | 5,855,608| 5,407,960  | 5,422,578  | 5,724,981| 5,430,135| 5,434,336  | 5,494,443|
| Coverage, x  | 58         | 49         | 196     | 44         | 44         | 37       | 24.8     | 40         | 42       |
| N50 value, bp| 128,340     | 136,532    | 247,851 | 219,102    | 126,261    | 162,973  | 71,482   | 179,463    | 106,056  |
| Genes        | 5812       | 5540       | 5845    | 5309       | 5121       | 5711     | 5526     | 5291       | 5535     |

Antimicrobial resistance genetic determinants

| Beta-Lactams          | \( \text{bla}_{\text{SHV}} \) | \( \text{bla}_{\text{TEM-1B}} \) | \( \text{bla}_{\text{CTX-M-15}} \) | \( \text{bla}_{\text{OXA-1}} \) | \( \text{bla}_{\text{OXA-48}} \) | \( \text{bla}_{\text{CTX-M-48}} \) | \( \text{bla}_{\text{CTX-M-15}} \) | \( \text{bla}_{\text{OXA-1}} \) | \( \text{bla}_{\text{OXA-48}} \) |
|-----------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| \( \text{SHV-182} \)  | \( \text{TEM-1B} \) | \( \text{CTX-M-15} \) | \( \text{OXA-1} \) | \( \text{CTX-M-15} \) | \( \text{OXA-48} \) | \( \text{CTX-M-48} \) | \( \text{CTX-M-15} \) | \( \text{OXA-1} \) | \( \text{OXA-48} \) |
Table 4. Cont.

| Isolate | B1396/18-2 | B1120K/18 | B14/19 | B1406/18-1 | B1230/18-1 | B1207/18 | B2523/18 | B1214/18-2 | B102/19 |
|----------|------------|------------|--------|------------|------------|-----------|----------|------------|---------|
| Aminoglycosides | ant(2')-Ia |aac(6')-Ib-cr|aadA1|ant(2')-Ia|aac(6')-Ib-cr|aadA2|aph(3')-Ib|aph(3')-Vla|aadA5|
| Fosfomycin | fosA |fosA |fosA |fosA |fosA |fosA |fosA |fosA |fosA |
| Phenics | catA1 |catB3 |catA2 |catA3 |catA3 |catA3 |catB3 |catA1 |catA1 |
| Quinolones | aac(6')-Ib-cr |qnrS1 |qnrS1 |qnrS1 |qnrS1 |qnrB1 |aac(6')-Ib-cr |qnrB1 |aac(6')-Ib-cr |
| Sulphonamides | sul1 |sul2 |sul1 |sul1 |sul1 |sul2 |sul1 |sul1 |
| Tetracyclines | tet |tet |tet |tet |tet |tet |tet |tet |
| Trimethoprim | dfrA1 |dfrA12 |dfrA14 |dfrA17 |dfrA17 |
| Macrolides | mph |
| Efflux | acr |env |fis |mar |oxy |ram |sdi |sdr |sal |sex |
| Virulence genetic determinants (loci) | | | | | | | | | | |
| Type 3 adhesin | mrk |
| Yersiniabactin biosynthesis | irp |
| Yersiniabactin transcriptional regulator | ybt |
| Siderophore yersiniabactin receptor | fyu |
| Aerobactin siderophore synthesis | iuc |
| Ferric aerobactin receptor | int |
| Regulator of capsular polysaccharide synthesis | kvg |
| Plasmids | IncFIB |IncHI1B |IncR |
| Abbreviations: pco, encoded copper (Cu) resistance; pbr, lead (Pb) resistance; sil, silver (Ag) resistance; ars, arsenic (As) resistance; ter, tellurium (Te) resistance. |

Analysis of virulence genetic determinants revealed mrk gene coding type 3 adhesine—in all 9 genomes, irp gene of yersiniabactin biosynthesis and ybt gene of yersiniabactin transcriptional regulator—in 7 genomes, fyu gene of siderophore yersiniabactin receptor—in 6 genomes, iuc gene of ferric aerobactin receptor—in 6 genomes, iuc gene of aerobactin siderophore synthesis—in 5 genomes, kvg gene of capsular polysaccharide synthesis regulator—in 2 genomes. Generally, each genome contained 1–7 virulence genes. Plasmids of eight incompatibility groups (IncC, IncFIA, IncFIB, IncFII, IncHI1B, IncM1, IncM2, and IncR) were identified in the K. pneumoniae isolate genomes, specifically 1–3 plasmids per genome. Molecular systems protecting bacteria from the foreign DNA, Type I Restriction-Modification systems, were detected in four genomes, Type II systems in all nine genomes, but CRISPR-Cas systems were not detected in the genomes (Table 4).

3. Discussion

K. pneumoniae isolates that caused severe postoperative infections in patients of neuro-ICU between 2017 and 2019 were divided into two groups, A and B, depending on observed clinical manifestations: associated and not associated with the pronounced systemic in-
Inflammation response. Previously, the association of *K. pneumoniae* with severe systemic infections was described as the modern trend for ICU; for example, *K. pneumoniae* were the most frequent infecting species (47%) determined meningitis/encephalitis and 30-day mortality rates, 15% in post-neurosurgical patients [12]. In our study, as in the reports from other countries, the overwhelming majority of *K. pneumoniae* isolates obtained from neurosurgery patients were MDR and XDR [13]. Moreover, MDR and XDR *K. pneumoniae* isolates in our study were associated with severe systemic infections, including vasculitis and microcirculation disorders. It was reported previously that *K. pneumoniae* was associated with leukocytoclastic vasculitis [4–6], rapidly progressive retinal vasculitis [14], and acute vasculitis at respiratory infection [15]. In this study, we first identified *K. pneumoniae* of ST395 and ST307 as bacterial pathogen associated with vasculitis and microcirculation disorders.

A total of twelve sequence types and nine capsular types of *K. pneumoniae* were identified in this study, which possibly reflects a continuous influx of new genetic lineages into neuro-ICU from other hospitals and regions. The prevalent ST in the ICU was ST395 (16/41 isolates), which is similarly rated to that previously published in the studies from Poland, France, Italy, and Russia [16–20]. Nine STs in our study (ST14, ST23, ST39, ST76, ST86, ST218, ST219, ST268, and ST2674) were identified only for the isolates that caused severe bloodstream infections. Previously *K. pneumoniae* of ST23, ST86, ST76, and ST218 were described as a hypervirulent pathogen causing bacteremia, sepsis, and liver abscess in India, France, China, Taiwan, and Russia [21–25]. Three STs (ST14, ST39, and ST268) in our study were associated with fatal outcomes. These STs have been reported previously as the agents of severe bloodstream infections in ICU and surgery wards in other countries [26,27]. Two sequence types, ST219, and ST2674 were first identified in this study as the agent of severe sepsis in the patients of ICU. Recently, the ST219 was reported for the environmental MDR *K. pneumoniae* strains collected from hospital wastewater in Southern Romania [28]; the ST2674 was identified for the environmental isolate from Pakistan (https://bigsdb.pasteur.fr/cgi-bin/bigsdb/bigsdb.pl?page=info&db=pubmlst_klebsiella_isolates&id=5256 access date 8 August 2021).

The high prevalence of polyresistant isolates in our study was associated with antimicrobial resistance genes. The isolates of Group A carried ESBL genes *bla*$_{CTX-M}$ (22/29), carbapenemase genes *bla*$_{OXA-48}$ (12/29), and *bla*$_{KPC-2}$ (1/29), which correspond to the reports from Greece and China [27,29,30]. Moreover, four isolates in Group A carrying the only *bla*$_{SHV}$ were identified (ST218 and ST86). These isolates, as well as four additional isolates of ST268, ST23, and ST76, carried virulence gene *rmpA* coding regulator of hypermucoid phenotype. It was reported previously that overexpression of *rmpA* could enhance the virulence of *K. pneumoniae* isolates in the mouse model [31]. Virulence genes *iroN* and *iroD* associated with utilization of trivalent iron were detected in *K. pneumoniae* of ST23, ST218, ST76, ST86, and ST268 (isolates associated with severe bloodstream infections) which agrees with reports from other countries [22,23,29]. It is known that the majority of pathogenic bacteria including *K. pneumoniae* possess the iron-acquisition system with a higher affinity for iron than the host, which serves as one of the strategies for increasing bacterial virulence [32]. The *kfu* gene coding iron uptake system was identified in *K. pneumoniae* of ST14 and ST219; the latter is a novel genetic lineage carrying the *kfu* gene [33,34]. *Kfu* was shown to be a potential virulence factor in the intragastrical murine model, which indicates that *kfu* might contribute to intestinal colonization [35]. Therefore, the identified virulence genes indicate the high potential of studied isolates for hypervirulence.

The isolates that were not associated with severe manifestations of systemic infections in our study were attributed to ST395, ST307, and ST147. Among them, the isolates of ST395 carrying the *bla*$_{OXA-48}$ carbapenemase gene were prevalent. Such *K. pneumoniae* strains were reported earlier from Hungary and Russia [36,37]. In our study, one isolate of ST147 carried the *bla*$_{NDM-1}$ carbapenemase gene, and the same strains have been described worldwide [38,39]. It should be noted that ST307 and ST147 have been estimated as *K. pneumoniae* High-Risk Clones (HRC) because of worldwide distribution, ability to cause serious infections, and association with polyresistance [40].
The whole-genome study of selected \textit{K. pneumoniae} isolates belonged to nine sequence types, ST23, ST39, ST76, ST86, ST218, ST219, ST307, ST395, and ST2674, showing the great diversity of these isolates in the combination of virulence genes, antimicrobial resistance genes, heavy metal resistance, and plasmids. Analysis of their resistomes showed that the genes of beta-lactamases \textit{bla}_{CTX-M-15}, \textit{bla}_{TEM-1B}, \textit{bla}_{NDM-1}, \textit{bla}_{OXA-48}, \textit{bla}_{KPC-2}, and \textit{bla}_{OXA-1} are represented by a single allele. These alleles were recently reported for \textit{K. pneumoniae} isolated in Russia [41]. On the contrary, six alleles were identified of \textit{bla}_{SHV}-type genes, which were not reported in Russia before our study: \textit{bla}_{SHV-26}, \textit{bla}_{SHV-28}, \textit{bla}_{SHV-33}, \textit{bla}_{SHV-40}, \textit{bla}_{SHV-59}, and \textit{bla}_{SHV-182}. In general, all isolates in our work carried \textit{bla}_{SHV} genes, 68\% isolates—\textit{bla}_{CTX-M} genes, 51\%—\textit{bla}_{TEM} genes, 51\%—\textit{bla}_{OXA-48} genes, but only one isolate carried \textit{bla}_{NDM-1} gene, and only one isolate \textit{bla}_{KPC-2} gene. A similar representation of beta-lactamase genes was reported from the European countries and Russia [41,42]. Major isolates in our study were susceptible to amikacin and imipenem, which is consistent with recently published data from Saudi Arabia and Indonesia [43,44]. Interestingly, the \textit{rmtB} gene encoding 16S rRNA methylase providing resistance to aminoglycosides in the \textit{K. pneumoniae} ST23 isolate was detected in this study for the first time. This gene was reported earlier for ST258 and ST16 of KPC-producing \textit{K. pneumoniae} [45]. Moreover, \textit{armA} gene coding 16S rRNA methyltransferase was detected in \textit{K. pneumoniae} of ST395 in this study; recently, this gene was detected in \textit{K. pneumoniae} ST23, ST2502, and ST11 in Italy, Spain, and China, respectively [46,47].

The virulence determinants detected in the genomes were the following: mannose-resistant \textit{Klebsiella}-like (mrk) hemagglutinin gene critical for \textit{K. pneumoniae} biofilm development in all 9 genomes; aerobactin siderophore locus (\textit{iuc}, \textit{iut}) in 6 genomes; regulator of capsular polysaccharide synthesis (\textit{kvg}) in 2 isolates. A similar distribution of virulence genes was reported recently from Brazil [48]. The yersiniabactin locus (\textit{irp}, \textit{ybt}, and \textit{fyu}) was detected in 7/9 genomes in our study, compared with 40\% of \textit{K. pneumoniae} genomes, particularly amongst those associated with invasive infections [49]. This data confirmed the high virulence potential of the studied clinical isolates. The presence in the genomes of the plasmids of different incompatibility groups indicates a possible role of ‘hybrid’ plasmids in the formation of \textit{K. pneumoniae} strains, simultaneously carrying a large number of antibiotic resistance and virulence genes. [9,50].

Future research will focus on studying the structure of plasmids carrying genes for antimicrobial resistance and virulence, as well as the expression of these genes under conditions of different genetic environments and selective pressure of antibiotics. We believe that further study of the microbiology, molecular biology, physiology, and interactions with the host of \textit{K. pneumoniae} will provide important knowledge to control \textit{K. pneumoniae} infection in ICUs.

4. Materials and Methods

4.1. Bioethical Requirements and Patients

Each patient signed informed voluntary consent to treatment and laboratory examination, under the requirements of the Russian Federation Bioethical Committee. The study did not contain personal data of patients; the clinical bacterial isolates information did not include name, date of birth, address, and disease history. The study was a retrospective observational study. The study was done in the neuro-ICU department in a specialized Neurosurgical Hospital in Moscow, Russia, with 300 beds that care for approximately 8000 patients per year, 95\% of whom undergo surgery. The surveillance software was designed in-house and integrated into the hospital’s electronic health record system [51]. Four types of health-associated infections were surveilled: bloodstream, respiratory and urinary tract infections, and healthcare-associated ventriculitis and meningitis [5].

4.2. Bacterial Isolates and Growing

\textit{K. pneumoniae} isolates (\textit{n} = 41) were collected from clinical samples (endotracheal aspirate, cerebrospinal fluid, blood, wound exudate, and urine) of the patients (\textit{n} = 20) in
the Neuro-ICU in the period from October 2017 to January 2019. Bacterial identification was performed using a Vitek-2 Compact (BioMerieux, Paris, France) and a MALDI-TOF Biotyper (Buker Daltonics, Bremen, Germany) instruments. Bacterial isolates were grown at 37 °C on Nutrient Medium No. 1 (SRCAMB, Obolensk, Moscow region, Russia), Luria-Bertani broth (Difco Laboratories, Detroit, MI, USA), and Muller-Hinton broth (Himedia, Mumbai, Maharashtra, India). Bacterial isolates were stored in 15% glycerol at minus 80 °C.

4.3. Antimicrobial Susceptibility

Susceptibility to 20 antimicrobials (AMs) of 8 functional groups: beta-lactams (ampicillin, ampicillin-sulbactam, cefuroxime, cefoxitin, ceftazidime, cefoperazone-sulbactam, cefepime, erapenem, imipenem), tetracyclines (tetracycline, tigecycline), fluoroquinolones (ciprofloxacin), phenicols (chloramphenicol), aminoglycosides (gentamicin, tobramycin, amikacin), sulfonamides (trimethoprim-sulfamethoxazole), nitrofurans (nitrofurantoin), and polymyxins (colistin) were determined using Vitek-2 Compact (BioMerieux, Paris, France). The results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing, Version 11.0, 2021 (http://www.eucast.org access date 8 August 2021). Reference strains Escherichia coli ATCC 25922 and ATCC 35218 were used as quality controls. The isolates were categorized as resistant (R), multidrug-resistant (MDR), or extensively drug-resistant (XDR) according to the criteria proposed by Magiorakos et al. [11].

4.4. Detection of Antimicrobial Resistance Genes

Beta-lactamase genes \( \text{bla}_{\text{SHV}}, \text{bla}_{\text{CTX-M}}, \text{bla}_{\text{TEM}}, \text{bla}_{\text{OXA-48}}, \text{bla}_{\text{KPC}}, \text{bla}_{\text{VIM}}, \text{bla}_{\text{IMP}}, \) and \( \text{bla}_{\text{NDM}}, \) and class 1 and 2 integrons were detected by PCR using previously described specific primers [52–60].

4.5. Detection of Virulence Genes and Capsular Type Identification

Eight genes associated with \( K. \) pneumoniae virulence, \( \text{rmpA} \) (hypermucoid phenotype regulator), \( \text{aer} \) (aerobactin), \( \text{kfu} \) (ferric absorption system), \( \text{uge} \) (uridine diphosphate-galacturonate-4-epimerase), \( \text{wabG} \) (glucosyltransferase), \( \text{fimH} \) (fimbria type I), \( \text{allS} \) and \( \text{allR} \) (allantoin regulon), were detected by PCR using specific primers [35,61–63]. The capsular serotypes of the \( K. \) pneumoniae isolates were determined by \( \text{wzi} \) gene amplification using specific primers [64], sequencings, and allele identification using the Institute Pasteur, Paris, France, BIGS database (https://bigsdb.pasteur.fr/ access date 8 August 2021).

4.6. Multilocus Sequence Typing

Sequence types (STs) of \( K. \) pneumoniae isolates were identified based on allelic profiles of seven housekeeping genes (\( \text{rpoB}, \text{gapA}, \text{mdh}, \text{pgi}, \text{phoE}, \text{infB}, \) and \( \text{tonB} \)), according to the Institute Pasteur, Paris, France, BIGS database protocol (https://bigsdb.pasteur.fr/klebsiella/primers_used.html access date 8 August 2021). Sequencing of DNA fragments was carried out at the SINTOL Center for collective use (Moscow, Russia). DNA sequences were analyzed using Vector NTI9 (Invitrogen, Carlsbad, CA, USA) and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi access date 8 August 2021).

4.7. Phylogenetic Analysis

The phylogenetic tree of \( K. \) pneumoniae STs was constructed using a web resource NCBI “Blastn” and “Blast Tree View” (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome access date 8 August 2021), based on combined gene sequences of MLST profiles.

4.8. Whole-Genome Sequencing

Whole-genome sequencing was done on the Illumina MiSeq platform using Nextera DNA Library Preparation Kit (Illumina, Carlsbad, CA, USA) and MiSeq Reagent Kits v3 (Illumina, Carlsbad, CA, United States). The obtained single reads were collected into
contigs using the SPAdes 3.9.0 software (Petersburg State University, St-Petersburg, Russia). De novo assembled genomes were annotated in the GenBank database (https://github.com/ncbi/pgap access date 8 August 2021). AM resistance genes, STs, and plasmids were identified using the web resources of the Center for Genomic Epidemiology: ResFinder, KmerResistance (90% identity threshold and 10% threshold for depth corr.), MLST, and PlasmidFinder (95% threshold for minimum identity and 60% minimum coverage) (http://www.genomecidepidemiology.org/ access date 8 August 2021). Virulence genes, capsular type, and efflux pumps were identified by the Institut Pasteur, Paris, France, BGIS database web-resource of (https://bigdb.pasteur.fr/ access date 8 August 2021).

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/antibiotics10080879/s1, Table S1: Minimal inhibitory concentrations (MICs) of antimicrobials for *K. pneumoniae* clinical isolates.

**Author Contributions:** Conceptualization, N.K.F., O.N.E. and I.A.D.; methodology, E.I.A., I.A.A., I.A.S.; software, A.A.K., M.V.F.; validation, T.S.N., M.V.F. and E.S.K.; formal analysis, O.N.E., N.K.F., S.F.B.; investigation, I.A.A., E.I.A., T.S.N., G.N.F., A.A.K., E.S.K.; resources, A.A.K., M.V.F.; data curation, A.A.K., M.V.F.; writing—original draft preparation, N.K.F., E.I.A.; writing—review and editing, O.N.E.; visualization, I.A.S., E.I.A.; supervision, N.K.F., O.N.E.; project administration, S.F.B., I.A.D.; funding acquisition, I.A.D. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Ministry of Science and Higher Education of the Russian Federation, grant number 075-15-2019-1671 (agreement dated 31 October 2019).

**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki, the study was designed as a retrospective observational study.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** Following Whole genome sequences were deposited in the GenBank database: JAGRZJ000000000, JAGRZI000000000, JAFJ000000000, JAGRZH000000000, JAFJ000000000, JAGRZG000000000, JAGRZF000000000, JAGRZE000000000, and JAFFJJ000000000.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Effah, C.Y.; Sun, T.; Liu, S.; Wu, Y. *Klebsiella pneumoniae*: An increasing threat to public health. *Ann. Clin. Microbiol. Antimicrob.* 2020, 19, 1–9. [CrossRef]

2. Zhang, S.; Yang, Z.; Sun, L.; Wang, Z.; Sun, L.; Xu, J.; Zeng, L.; Sun, T. Clinical Observation and Prognostic Analysis of Patients With *Klebsiella pneumoniae* Bloodstream Infection. *Front. Cell. Infect. Microbiol.* 2020, 10, 577244. [CrossRef]

3. Ershova, K.; Savin, I.; Kudryumova, N.; Wong, D.; Danilov, G.; Shifrín, M.; Alexandrova, I.; Sokolova, E.; Fursova, N.; Zelman, V.; et al. Implementing an infection control and prevention program decreases the incidence of healthcare-associated infections and antibiotic resistance in a Russian neuro-ICU. *Antimicrob. Resist. Infect. Control.* 2018, 7, 94. [CrossRef]

4. Lum, P.N.; Woo, P.C.Y.; Wong, S.S.; Yuen, K.-Y. *Klebsiella pneumoniae* bacteremia. *Diagn. Microbiol. Infect. Dis.* 2000, 37, 275–277. [CrossRef]

5. Lloret, P.; Redondo, P.; Molano, E. *Klebsiella pneumoniae* and leukocytoclastic vasculitis. *Lancet* 2002, 360, 1062. [CrossRef]

6. Huang, H.Y.; Wu, Y.-H.; Kuo, C.F. *Klebsiella pneumoniae* sepsis with unusual cutaneous presentation of generalized pustulosis. *Clin. Exp. Dermatol.* 2013, 38, 626–629. [CrossRef]

7. Wyres, K.L.; Wick, R.R.; Judd, L.M.; Froumine, R.; Tokolyi, A.; Gorrie, C.L.; Lam, M.M.C.; Duchêne, S.; Jenney, A.; Holt, K. Distinct evolutionary dynamics of horizontal gene transfer in drug resistant and virulent clones of *Klebsiella pneumoniae*. *PLoS Genet.* 2019, 15, e1008114. [CrossRef] [PubMed]

8. El-Domany, R.A.; Awadalla, O.A.; Shabana, S.A.; El-Dardir, M.A.; Emara, M. Analysis of the Correlation Between Antibiotic Resistance Patterns and Virulence Determinants in Pathogenic *Klebsiella pneumoniae* Isolates from Egypt. *Microb. Drug Resist.* 2021, 27, 727–739. [CrossRef]

9. Lan, P.; Jiang, Y.; Zhou, J.; Yu, Y. A global perspective on the convergence of hypervirulence and carbapenem resistance in *Klebsiella pneumoniae*. *J. Glob. Antimicrob. Resist.* 2021, 25, 26–34. [CrossRef] [PubMed]

10. Yu, W.-L.; Ko, W.-C.; Cheng, K.-C.; Lee, C.-C.; Lai, C.-C.; Chuang, Y.-C. Comparison of prevalence of virulence factors for *Klebsiella pneumoniae* liver abscesses between isolates with capsular K1/K2 and non-K1/K2 serotypes. *Diagn. Microbiol. Infect. Dis.* 2008, 62, 1–6. [CrossRef]
11. Magiorakos, A.-P.; Srinivasan, A.; Carey, R.B.; Carmeli, Y.; Falagas, M.E.; Giske, C.; Harbarth, S.; Hindler, J.F.; Kahlmeter, G.; Olsson-Liljequist, B.; et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* 2012, 18, 268–281. [CrossRef]

12. Shi, Y.-J.; Zheng, G.-H.; Qian, L.-Y.; Qsman, R.A.; Li, G.-G.; Zhang, G.-J. Longitudinal Analysis of Risk Factors for Clinical Outcomes of Enterobacteriaceae Meningitis/Encephalitis in Post-Neurosurgical Patients: A Comparative Cohort Study During 2014–2019. *Infect. Drug Resist.* 2020, 13, 2161–2170. [CrossRef]

13. Yang, W.; Wu, X.; Li, Z.; Yuan, Q.; Wu, G.; Yu, J.; Wu, X.; Du, Z.; Hu, J.; Zhou, L. Trends of Intra-Cranial Bacterial Infection in Patients Requiring Emergency Neurosurgery. *Surg. Infect.* 2020, 21, 677–683. [CrossRef] [PubMed]

14. Jin, K.W.; Kim, E.; Kim, H.; Bae, S.H. Klebsiella Endophthalmitis as Retinal Vasculitis with Prostatic Abscess. *Optom. Vis. Sci.* 2015, 92, e158–e160. [CrossRef]

15. Castan, P.; Maigne, G.; Boutemy, J.; Silva, N.M.; De Boysson, H.; Aouba, A.; Audemard-Verger, A. Vascularite à IgA satellite d’une pneumopathie abécédée à *Klebsiella pneumoniae* IgA vasculitis secondary to *Klebsiella pneumoniae* infection. *Rev. Mal. Respir.* 2020, 37, 417–421. [CrossRef]

16. Izdebski, R.; Baraniak, A.; Žabicka, D.; Machulska, M.; Urbanowicz, P.; Fiett, J.; Literacka, E.; Bojarska, K.; Kozzińska, A.; Zieniuk, B.; et al. Enterobacteriaceae producing OXA-48-like carbapenemases in Poland, 2013–2017. *J. Antimicrob. Chemother.* 2017, 73, 620–625. [CrossRef]

17. Muggeo, A.; Guillard, T.; Klein, F.; Reffuveille, F.; François, C.; Babosan, A.; Bajolet, O.; Bertrand, X.; de Champs, C. Spread of *Klebsiella pneumoniae* ST395 non-susceptible to carbapenems and resistant to fluoroquinolones in North-Eastern France. *J. Glob. Antimicrob. Resist.* 2018, 13, 98–103. [CrossRef] [PubMed]

18. Maida, C.M.; Bonura, C.; Geraci, D.M.; Graziano, G.; Carattoli, A.; Rizzo, X.; Torregrossa, M.V.; Vecchio, D.; Giuffré, M. Outbreak of ST395 KPC-Producing *Klebsiella pneumoniae* in a Neonatal Intensive Care Unit in Palermo, Italy. *Infect. Control. Hosp. Epidemiol.* 2018, 39, 496–498. [CrossRef] [PubMed]

19. Kuptsov, N.; Kornienko, M.; Gorodnichev, R.; Danilov, D.; Malakhova, M.; Parfenova, T.; Makarenko, G.; Shitikov, E.; Iлина, E. Efficacy of commercial bacteriophage products against ESRAPE pathogens. *Bull. Russ. St. Med. Univer.* 2020, 3, 18–24. [CrossRef]

20. Fursova, N.K.; Astashkin, E.I.; Gabrielyan, N.I.; Novikova, T.S.; Fedyukina, G.N.; Kubanova, M.K.; Esenova, N.M.; Sharapchenko, S.O.; Volozhantsev, N.V. Emergence of Five Genetic Lines ST395NDM-1, ST13OXA-48, ST3346OXA-48, ST39CTX-M-14, and Novel ST3551OXA-48 of Multidrug-Resistant Clinical *Klebsiella pneumoniae* in Russia. *Microb. Drug Resist.* 2020, 26, 924–933. [CrossRef]

21. Shankar, C.; Jacob, J.J.; Vasudevan, K.; Biswas, R.; Manesh, A.; Sethuvel, D.P.M.; Varughese, S.; Biswas, I.; Veeraraghavan, B. Emergence of Multidrug Resistant Hypervirulent ST23 *Klebsiella pneumoniae*: Multidrug Resistant Plasmid Acquisition Drives Evolution. *Front. Cell. Infect. Microbiol.* 2020, 10, 575289. [CrossRef] [PubMed]

22. Baron, S.A.; Pascale, L.-M.; Million, M.; Briandais, A.; Durand, J.-M.; Hadjadj, L.; Rolain, J.-M. Whole genome sequencing to decipher the virulence phenotype of hypervirulent *Klebsiella pneumoniae* responsible for liver abscesses, Marseille, France. *Eur. J. Clin. Microbiol. Infect. Dis.* 2021, 40, 1073–1077. [CrossRef]

23. Su, S.; Li, C.; Zhao, Y.; Yu, L.; Wang, Y.; Wang, Y.; Bao, M.; Fu, Y.; Zhang, J.; Zhang, X. Outbreak of KPC-2-Producing *Klebsiella pneumoniae* ST76 Isolates in an Intensive Care Unit and Neurosurgery Unit. *Microb. Drug Resist.* 2020, 26, 1009–1018. [CrossRef]

24. Liao, C.-H.; Huang, Y.T.; Chang, C.Y.; Hsu, H.S.; Hsueh, P.R. Capsular serotypes and multilocus sequence types of bacteremic *Klebsiella pneumoniae* isolates associated with different types of infections. *Eur. J. Clin. Microbiol. Infect. Dis.* 2013, 33, 365–369. [CrossRef] [PubMed]

25. Lev, A.I.; Astashkin, E.I.; Kislichkina, A.A.; Solovieva, E.V.; Kombarova, T.I.; Korobova, O.V.; Ershova, O.N.; Alexandrova, I.A.; Malikov, V.E.; Bogun, A.G.; et al. Comparative analysis of *Klebsiella pneumoniae* strains isolated in 2012–2016 that differ by antibiotic resistance genes and virulence gene profiles. *Pathog. Glob. Health* 2018, 112, 142–151. [CrossRef] [PubMed]

26. Piccirilli, A.; Perilli, M.; Piccirilli, V.; Segatore, B.; Amicosante, G.; Maccaraco, L.; Bazzai, A.; Naso, L.; Cascio, G.L.; Cornaglia, G. Molecular characterization of carbapenem-resistant *Klebsiella pneumoniae* ST14 and ST512 causing bloodstream infections in ICU and surgery wards of a tertiary university hospital of Verona (northern Italy): Co-production of KPC-3, OXA-48, and CTX-M-15 β-lactamases. *Diagn. Microbiol. Infect. Dis.* 2020, 96, 114968. [CrossRef]

27. Galani, I.; Karaiskos, I.; Angelidis, E.; Papoutsaki, V.; Galani, L.; Souli, M.; Antoniadou, A.; Giamarelou, H. Emergence of ceftazidime-avibactam resistance through distinct genomic adaptations in KPC-2-producing *Klebsiella pneumoniae* sequence type 39 during treatment. *Eur. J. Clin. Microbiol. Infect. Dis.* 2021, 40, 219–224. [CrossRef]

28. Surleac, M.; Barbu, I.C.; Parasciv, S.; Popa, L.I.; Gheorghe, I.; Marutescu, L.; Popa, M.; Sarbu, I.; Talapan, D.; Nita, M.; et al. Whole genome sequencing snapshot of multi-drug resistant *Klebsiella pneumoniae* strains from hospitals and receiving wastewater treatment plants in Southern Romania. *PLoS ONE* 2020, 15, e0228079. [CrossRef]

29. Shen, P.; Berglund, B.; Chen, Y.; Zhou, Y.; Xiao, T.; Xiao, Y.; Zhou, K. Hypervirulence Markers Among Non-ST11 Strains of Carbapenem- and Multidrug-Resistant *Klebsiella pneumoniae* Isolated From Patients With Bloodstream Infections. *Front. Microbiol.* 2020, 11, 1199. [CrossRef]

30. Xie, S.; Fu, S.; Li, M.; Guo, Z.; Zhu, X.; Ren, J.; Hu, F. Microbiological Characteristics of Carbapenem-Resistant Enterobacteriaceae Clinical Isolates Collected from County Hospitals. *Infect. Drug Resist.* 2020, 13, 1163–1169. [CrossRef] [PubMed]
32. Zhu, J.; Wang, T.; Chen, L.; Du, H. Virulence Factors in Hypervirulent Klebsiella pneumoniae. Front. Microbiol. 2021, 12, 642484. [CrossRef] [PubMed]
33. Chen, Q.; Zhou, J.-W.; Qiu, C.-N.; Wang, M.-M.; Wang, X.-J.; Ruan, Z.; Fan, J.-Z.; Qiong, C.; Jia-Wei, Z.; Chun-Ning, Q.; et al. Antibiotics 2021 (47). High Prevalence of 16S rRNA Methylase Genes in a tertiary hospital in Hangzhou, China. J. Glob. Antimicrob. Resist. 2018, 15, 61–64. [CrossRef]
34. Nava, R.G.; Oliveira-Silva, M.; Nakamura-Silva, R.; Pitoondo-Silva, A.; Vespero, E.C. New sequence type in multidrug-resistant Klebsiella pneumoniae harboring the blaNDM-1-encoding gene in Brazil. Int. J. Infect. Dis. 2019, 79, 101–103. [CrossRef]
35. Ma, L.-C.; Fang, C.-T.; Lee, C.-Z.; Shun, C.-T.; Wang, J.-T. Genomic Heterogeneity in Klebsiella pneumoniae Strains Is Associated with Primary Pyogenic Liver Abscess and Metastatic Infection. Infect. Dis. 2005, 192, 117–128. [CrossRef]
36. Kovács, K.; Nyul, A.; Mestyán, G.; Melegh, S.; Fenyvesi, H.; Jakab, G.; Szabó, H.; Jányvári, L.; Damjanova, I.; Tóth, Á. Emergence and interhospital spread of OXA-48-producing Klebsiella pneumoniae ST395 clone in Western Hungary. Infect. Dis. 2016, 49, 231–233. [CrossRef]
37. Alekseeva, E.A.; Brusnigina, N.F.; Solntsev, A.L.; Gordinskaya, A.N. The molecular typing of clinical isolates Klebsiella pneumoniae producing beta-lactamases of extended specter of action. Klin. Lab. Diagn. 2017, 62, 699–704.
38. Wu, W.; Feng, Y.; Tang, G.; Qiao, F.; McNally, A.; Zong, Z. NDM Metallo-β-Lactamas and Their Bacterial Producers in Health Care Settings. Clin. Microbiol. Rev. 2019, 32, e00115-18. [CrossRef] [PubMed]
39. Arena, F.; Di Pilato, V.; Vannetti, F.; Fabbri, L.; Antonelli, A.; Coppí, M.; Pupillo, R.; Macchi, C.; Rossolino, G.M. Population structure of KPC carbapenemase-producing Klebsiella pneumoniae in a long-term acute-care rehabilitation facility: Identification of a new lineage of clonal group 101, associated with local hyperendemicity. Microb. Genom. 2020, 6, e000308. [CrossRef] [PubMed]
40. Peirano, G.; Chen, L.; Kreiswirth, B.N.; Pitout, J.D.D. Emerging Antimicrobial-Resistant High-Risk Klebsiella pneumoniae Clones ST307 and ST147. Antimicrob. Agents Chemother. 2020, 64, e01148-20. [CrossRef] [PubMed]
41. Skachkova, T.; Shipulina, O.; Shipulin, G.; Shelenkov, A.; Yanushevich, Y.; Mikhailova, Y.; Zamytin, M.; Gusarov, V.; Petrova, N.; Lashenkova, N.; et al. Characterization of genetic diversity of the Klebsiella pneumoniae strains in a Moscow tertiary care center using next-generation sequencing. Clin. Microbiol. Antimicrob. Chemother. 2019, 21. [CrossRef]
42. Lee, C.-R.; Lee, J.H.; Park, K.S.; Kim, Y.B.; Jeong, B.C.; Lee, S.H. Global Dissemination of Carbapenemase-Producing Klebsiella pneumoniae: Epidemiology, Genetic Context, Treatment Options, and Detection Methods. Front. Microbiol. 2016, 7, 895. [CrossRef]
43. Azim, N.S.A.; Noñal, M.Y.; Alharbi, M.A.; Al-Zaban, M.I.; Somily, A. Molecular-diversity, Prevalence and Antibiotic Susceptibility of Pathogenic Klebsiella pneumoniae under Saudi Condition. Pak. J. Biol. Sci. 2019, 22, 174–179. [CrossRef]
44. Ishii, A.; Shigemura, K.; Kitagawa, K.; Harada, M.; Kan, Y.; Hayashi, F.; Osawa, K.; Kuntaman, K.; Shirakawa, T.; Fujisawa, M. Cross-Resistance and the Mechanisms of Cephalosporin-Resistant Bacteria in Urinary Tract Infections Isolated in Indonesia. Curr. Microbiol. 2021, 78, 1771–1777. [CrossRef] [PubMed]
45. Roch, M.; Sierra, R.; Sands, K.; Martins, W.M.; Schrenzel, J.; Walsh, T.R.; Gales, A.C.; Andrey, D.O. Vertical and horizontal dissemination of an IncC plasmid harbouring rmtB 16S rRNA methylase gene, conferring resistance to plazomicin, among invasive ST258 and ST16 KPC-producing Klebsiella pneumoniae isolates in a Chinese Tertiary Hospital. Microb. Drug Resist. 2021, 27, 44–52. [CrossRef]
46. Hernández, M.; López-Urrutia, L.; Abad, D.; Serna, M.D.F.; Ocampo-Sosa, A.; Eiros, J. First Report of an Extensively Drug-Resistant ST23 Klebsiella pneumoniae of Capsular Serotype K1 Co-Producing CTX-M-15, OXA-48 and ArmA in Spain. Antibiotics 2021, 10, 157. [CrossRef] [PubMed]
47. Liao, W.; De Wang, L.; Li, D.; Du, F.-L.; Long, D.; Liu, Y.; Ng, O.; Zhang, W. High Prevalence of 16s rRNA Methylase Genes Among Carbapenem-Resistant Hypervirulent Klebsiella pneumoniae Isolates in a Chinese Tertiary Hospital. Microb. Drug Resist. 2021, 27, 44–52. [CrossRef]
48. Nakamura-Silva, R.; Oliveira-Silva, M.; Furlan, J.P.R.; Stehling, E.G.; Miranda, C.E.S.; Pitoondo-Silva, A. Characterization of multidrug-resistant and virulent Klebsiella pneumoniae strains belonging to the high-risk clonal group 258 (CG258) isolated from inpatients in northeastern Brazil. Arch. Microbiol. 2021, 203, 4351–4359. [CrossRef]
49. Lam, M.M.C.; Wick, R.R.; Wyres, K.L.; Gorrie, C.L.; Judd, L.M.; Jenney, A.W.J.; Brisse, S.; Holt, K.E. Genetic diversity, mobilisation and spread of the yersiniabactin-encoding mobile element ICKEp in Klebsiella pneumoniae populations. Microb. Genom. 2018, 4, e00196. [CrossRef] [PubMed]
50. Rodriguez-Navarro, J.; Miró, E.; Brown-Jaque, M.; Hurtado, J.C.; Moreno, A.; Muniesa, M.; González-López, J.J.; Vila, J.; Espinal, P.; Navarro, F. Comparison of Commensal and Clinical Isolates for Diversity of Plasmids in Escherichia coli and Klebsiella pneumoniae. Antimicrob. Agents Chemother. 2020, 64, e02064-19. [CrossRef]
51. Shifrin, M.; Kurdumova, N.; Danilov, G.; Ershova, O.; Savin, I.; Alexandrova, I.; Sokolova, E.; Tabasaranisky, T. Electronic patient records system as a monitoring tool. Stud. Heal. Technol. Inform. 2015, 210, 236–238. [CrossRef]
52. Eckert, C.; Gautier, Y.; Arlet, G. DNA sequence analysis of the genetic environment of various blaCTX-M genes. J. Antimicrob. Chemother. 2005, 57, 14–23. [CrossRef]
53. Edelstein, M.; Pimkin, M.; Palagin, I.; Strachounski, L. Prevalence and Molecular Epidemiology of CTX-MExtended-Spectrum β-Lactamate-Producing Escherichia coli and Klebsiella pneumoniae in Russian Hospitals. Antimicrob. Agents Chemother. 2003, 47, 3724–3729. [CrossRef]
54. Priamchuk, S.D.; Fusurova, N.K.; Abaev, I.; Kovalev, I.N.; Shishkova, A.N.; Pecherskikh, I.E.; Korobova, O.V.; Astashkin, I.E.; Pachkunov, D.M.; Kruglov, A.N.; et al. Genetic determinants of antibacterial resistance among nosocomial Escherichia coli, Klebsiella spp., and Enterobacter spp. isolates collected in Russia within 2003–2007. Antibi. Khimioter. 2010, 55, 3–10. [PubMed]
55. Poirel, L.; Bonnin, R.A.; Nordmann, P. Genetic Features of the Widespread Plasmid Coding for the Carbapenemase OXA-48. *Antimicrob. Agents Chemother.* 2011, 56, 559–562. [CrossRef]
56. Rasheed, J.K.; Biddle, J.W.; Anderson, K.E.; Washer, L.; Chenuweth, C.; Perrin, J.; Newton, D.W.; Patel, J.B. Detection of the *Klebsiella pneumoniae* Carbapenemase Type 2 Carbapenem-Hydrolyzing Enzyme in Clinical Isolates of *Citrobacter freundii* and *K. oxytoca* Carrying a Common Plasmid. *J. Clin. Microbiol.* 2008, 46, 2066–2069. [CrossRef] [PubMed]
57. Hujer, K.M.; Hujer, A.M.; Hulten, E.A.; Bajaksouzian, S.; Adams, J.M.; Donskey, C.J.; Ecker, D.J.; Massire, C.; Eshoo, M.W.; Sampath, R.; et al. Analysis of Antibiotic Resistance Genes in Multidrug-Resistant *Acinetobacter* sp. Isolates from Military and Civilian Patients Treated at the Walter Reed Army Medical Center. *Antimicrob. Agents Chemother.* 2006, 50, 4114–4123. [CrossRef]
58. Martins, A.F.; Zavascki, A.P.; Gaspareto, P.B.; Barth, A.L. Dissemination of *Pseudomonas aeruginosa* Producing SPM-1-like and IMP-1-like Metallo-β-lactamases in Hospitals from Southern Brazil. *Infection* 2007, 35, 457–460. [CrossRef] [PubMed]
59. Yang, J.; Chen, Y.; Jia, X.; Luo, Y.; Song, Q.; Zhao, W.; Wang, Y.; Liu, H.; Zheng, D.; Xia, Y.; et al. Dissemination and characterization of NDM-1-producing *Acinetobacter pittii* in an intensive care unit in China. *Clin. Microbiol. Infect.* 2012, 18, E506–E513. [CrossRef]
60. Machado, E.; Cantón, R.; Baquero, F.; Galán, J.-C.; Rollán, A.; Peixe, L.; Coque, T.M. Integron Content of Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* Strains over 12 Years in a Single Hospital in Madrid, Spain. *Antimicrob. Agents Chemother.* 2005, 49, 1823–1829. [CrossRef] [PubMed]
61. Nassrf, X.; Honoré, N.; Vasselon, T.; Cole, S.T.; Sansonetti, P.J. Positive control of colanic acid synthesis in *Escherichia coli* by rpmA and rpmB, two virulence-plasmid genes of *Klebsiella pneumoniae*. *Mol. Microbiol.* 1989, 3, 1349–1359. [CrossRef] [PubMed]
62. Regué, M.; Hita, B.; Piqué, N.; Izquierdo, L.; Merino, S.; Fresno, S.; Benedi, V.J.; Tomás, J.M. A Gene, uge, Is Essential for *Klebsiella pneumoniae* Virulence. *Infect. Immun.* 2004, 72, 54–61. [CrossRef] [PubMed]
63. Izquierdo, L.; Coderch, N.; Piqué, N.; Bedini, E.; Corsaro, M.M.; Merino, S.; Fresno, S.; Tomás, J.M.; Regué, M. The *Klebsiella pneumoniae* wabG Gene: Role in Biosynthesis of the Core Lipopolysaccharide and Virulence. *J. Bacteriol.* 2003, 185, 7213–7221. [CrossRef] [PubMed]
64. Brisse, S.; Passet, V.; Hauaggaard, A.B.; Babosan, A.; Kassiss-Chikhani, N.; Struve, C.; Decré, D. wzi Gene Sequencing, a Rapid Method for Determination of Capsular Type for *Klebsiella* Strains. *J. Clin. Microbiol.* 2013, 51, 4073–4078. [CrossRef] [PubMed]