ORIGINAL ARTICLES
(CC BY-SA)

UDC: 616-022.7:614.2]:616.9-022.7-036
DOI: https://doi.org/10.2298/VSP251023011S

A retrospective cohort study of survival in patients with healthcare-associated infection caused by *Klebsiella pneumoniae* – clinical, phenotypic, and genotypic predictors in a tertiary healthcare institution in Serbia (2022–2023)

Retrospektivna kohortna studija preživljavanja bolesnika sa bolničkom infekcijom izazvanom bakterijom *Klebsiella pneumoniae* – klinički, fenotipski i genotipski prediktori u tercijarnoj zdravstvenoj ustanovi u Srbiji (2022–2023)

Marko Simonović*, Bojan Rakonjac†, Aleksandra Nikolić‡, Jelena Dabić†, Duško Maksimović§, Momčilo Djurić†, Djordje Taušan‖, Vesna Šuljagić¶**

Military Medical Academy, ‖Treatment Sector, *Institute of Epidemiology, †Institute of Microbiology, §Clinic for Anesthesiology and Critical Care, ¶Department of Healthcare-Related Infection Control, Belgrade, Serbia; ‡University of Belgrade, Faculty of Medicine, Belgrade, Serbia; **University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. *Klebsiella* (*K.*) *pneumoniae* is a frequent cause of healthcare-associated infections (HAI), particularly in intensive care units. Carbapenem-resistant strains represent a serious threat due to high mortality and limited therapeutic options. The aim of this study was to identify clinical predictors of 30-day mortality and to determine the presence of carbapenemase genes among *K. pneumoniae* isolates. **Methods.** A retrospective cohort study was conducted at the Military Medical Academy in Belgrade, Serbia. It included 121 patients with HAI caused by *K. pneumoniae* between January 2022 and December 2023. Clinical data were collected through active HAI surveillance. Isolation and antimicrobial susceptibility testing were performed according to standard microbiological procedures, and detection of carbapenemase genes was carried out using multiplex polymerase chain reaction. Survival was analyzed using the Kaplan-Meier method, and predictors of mortality were assessed using Cox regression analysis. **Results**. Thirty-day mortality was 59.5%. High resistance rates were observed to aminoglycosides (84.3%), fluoroquinolones (94.2%), and carbapenems (95.9%), while 67.8% of isolates were multidrug-resistant. The most common gene was $bla_{OXA\text{-}48\text{-}like}$ (45.5% in 2022 and 65.9% in 2023), followed by bla_{NDM} (22.7% in 2022 and 4.5% in 2023), while bla_{KPC} was detected only in isolates from 2023 (23.9%). The most frequently detected combination of carbapenemase genes was bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$ (31.8% in 2022 and 5.7% in 2023). Detected genes had no significant effect on survival. Age ≥ 70 years, bloodstream infection, and intensive care units stay were identified as independent predictors of 30-day mortality. **Conclusion**. The high mortality among patients with HAI caused by carbapenem-resistant *K. pneumoniae* strains was primarily associated with patient characteristics and disease severity rather than the presence of specific carbapenemase genes.

Keywords:
beta-lactamases; carbapenems; cross infection; drug resistance, bacterial; klebsiella pneumoniae; mortality; tertiary care centers.

Apstrakt

Uvod/Cilj. *Klebsiella* (*K.*) *pneumoniae* je čest uzročnik bolničkih infekcija (*healthcare-associated infections* – HAI), naročito u jedinicama intenzivne nege. Karbapenem-rezistentni sojevi predstavljaju ozbiljnu pretnju zbog visoke smrtnosti i ograničenih terapijskih mogućnosti. Cilj rada bio je da se identifikuju klinički prediktori 30-dnevne smrtnosti i da se utvrdi prisustvo gena za karbapenemaze kod izolata *K. pneumoniae*. **Metode**. Retrospektivna kohortna studija sprovedena je na Vojnomedicinskoj akademiji u Beogradu, Srbija. Studija je obuhvatila 121 bolesnika sa HAI izazvanim *K. Pneumoniae* od januara 2022. do decembra 2023. godine. Klinički podaci prikupljeni su aktivnim nadzorom nad HAI. Izolacija i ispitivanje osetljivosti na antimikrobne lekove urađeni su standardnim

Correspondence to: Marko Simonović, Military Medical Academy, Institute of Epidemiology, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: markosimonovic84@gmail.com

mikrobiološkim procedurama, a detekcija gena za karbapenemaze urađena je metodom multipleks lančane reakcije polimeraze. Preživljavanje je analizirano Kaplan-Majerovom metodom, a prediktori smrtnosti Koksovom regresionom analizom. **Rezultati**. Tridesetodnevna smrtnost iznosila je 59,5%. Zabeležene su visoke stope rezistencije na aminoglikozide (84,3%), fluorohinolone (94,2%) i karbapeneme (95,9%), dok je 67,8% izolata bilo multirezistentno. Najčešći gen bio je *bla*$_{OXA\text{-}48\text{-}like}$ (45,5% u 2022. i 65,9% u 2023. godini), zatim *bla*$_{NDM}$ (22,7% u 2022. i 4,5% u 2023. godini), dok je *bla*$_{KPC}$ (23,9%) detektovan samo u 2023. godini. Najčešće otkrivena kombinacija gena za karbapenemaze bila je *bla*$_{NDM}$ + *bla*$_{OXA\text{-}48\text{-}like}$ (31,8% u 2022. i 5,7% u 2023. godini). Detektovani geni nisu imali značajan uticaj na preživljavanje. Starost ≥ 70 godina, infekcija krvotoka i boravak u jedinicama intenzivne nege identifikovani su kao nezavisni prediktori 30-dnevne smrtnosti. **Zaključak**. Visoka smrtnost bolesnika sa HAI izazvanom karbapenem-rezistentnim sojevima *K. pneumoniae* pre svega je bila povezana sa karakteristikama bolesnika i težinom osnovnog oboljenja, a ne sa prisustvom gena za karbapenemaze.

Ključne reči:
beta-laktamaze; karbapenemi; infekcija, intrahospitalna; lekovi, rezistencija mikroorganizama; klebsiella pneumoniae; mortalitet; zdravstvene ustanove, tercijarne.

Introduction

Klebsiella (*K.*) *pneumoniae* is a major cause of healthcare-associated infections (HAIs), including bloodstream infections (BSIs), pneumonia, and urinary tract infections. It represents one of the leading pathogens in intensive care units (ICUs) worldwide [1–3]. The emergence and spread of carbapenem-resistant *K. pneumoniae* (CRKP) have become a significant global public health challenge due to limited therapeutic options and high associated mortality rates [4, 5]. The World Health Organization (WHO) has identified carbapenem-resistant Enterobacterales, particularly CRKP, as critical priority pathogens requiring urgent research and development of new treatment options [5].

The prevalence of CRKP infections varies geographically, with the highest rates observed in Southern and Eastern Europe [6–8]. Data from the European Center for Disease Prevention and Control (ECDC) and the Central Asian and European Surveillance of Antimicrobial Resistance highlight persistently high resistance levels in the Balkan region, including Serbia, where carbapenem resistance rates among invasive *K. pneumoniae* isolates exceed 62.7% [7, 8]. Local studies confirm that CRKP strains predominate in Serbian hospitals, reflecting the regional epidemiological situation [9, 10].

The strong tendency of *K. pneumoniae* to acquire genetic material *via* horizontal gene transfer has facilitated the emergence of multidrug-resistant (MDR) strains, which are now predominant in hospital settings [1, 3]. Carbapenem resistance is primarily driven by the production of carbapenemases, such as New Delhi metallo-β-lactamase (NDM), oxacillinase-48 (OXA-48), *K. pneumoniae* carbapenemase (KPC), Verona integron-encoded metallo-β-lactamase (VIM), and imipenemase (IMP), which can hydrolyze carbapenems and other β-lactams [1, 3]. Studies assessing whether carbapenemase type influences survival in CRKP infections have shown inconsistent results. Some suggest higher mortality with metallo-β-lactamase producers, while others find that host factors and illness severity are more predictive than enzyme type [11–15].

In addition to host-related vulnerabilities, environmental and organizational factors, such as adherence to infection prevention and control (IPC) measures, staffing levels, and variability in nursing training, play a critical role in the acquisition and outcomes of HAIs caused by *K. pneumoniae*. These factors are particularly relevant in high-MDR healthcare settings, where systemic constraints may facilitate the transmission of CRKP. Several studies, including large systematic reviews, have shown that lower nurse-staffing levels and higher workloads are associated with increased risk of HAI, underscoring the importance of adequate staffing, adherence to IPC protocols, and organizational support in preventing CRKP spread [16, 17].

The aim of this study was to identify risk factors and mortality outcomes associated with *K. pneumoniae* HAIs and to provide molecular characterization of carbapenemase-producing isolates collected in a tertiary healthcare institution in Serbia.

Methods

This retrospective cohort study included 121 patients with registered HAIs caused by *K. pneumonia* between January 2022 and December 2023. The study was conducted at the Military Medical Academy (MMA), Belgrade, Serbia, a teaching hospital affiliated with the University of Defence, Belgrade. The MMA is a 1,000-bed tertiary university healthcare center, divided into 27 departments according to medical specialty. The study was approved by the Ethics Committee of the Faculty of Medicine MMA (No. 5/7/2024, from April 4, 2024).

Surveillance of healthcare-associated infection

The Department of Healthcare-Related Infection Prevention and Control performs continuous HAI surveillance among ICU and surgical patients hospitalized for more than 48 hrs. Patients were visited daily by an infection control nurse and a physician for data collection. The following variables were collected: age, sex, type of infection (pneumonia, BSI, urinary tract infection, surgical site infection), surgery within 30 days, ICU admission, primary diagnosis (cardiovascular, gastrointestinal, neurological, respiratory, cancer, injuries/intoxications, other), McCabe classification, pres-

ence of invasive devices [drain, central venous catheter (CVC), mechanical ventilation (MV), urinary catheter (UC)], and outcome. The ECDC definitions for HAIs translated into Serbian were applied [18].

Isolation and identification of Klebsiella pneumoniae

Clinical samples were collected from hospitalized patients with HAIs and processed according to standard operating procedures, including inoculation on appropriate culture media and incubation for 18–24 hrs under aerobic conditions at 37 °C. Isolate identification to the species level was performed using MALDI-TOF MS (Vitek® MS, bioMérieux, France). Isolates grown under aerobic conditions for 18–24 hrs on blood agar plates with 5% sheep blood were applied onto the analysis plate using a sterile loop, air-dried, and overlaid with matrix solution (Vitek® MS-CHCA, bioMérieux). The calibration strain *Escherichia coli* ATCC® 8739™ was analyzed in parallel. Non-repetitive *K. pneumoniae* isolates were included in further analyses.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined using the disk diffusion method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodology and by minimum inhibitory concentration using an automated system (Vitek® 2, bioMérieux, France). Results were interpreted following EUCAST standards.

For the Kirby-Bauer disk diffusion method, Mueller-Hinton agar (pH 7.2–7.4) and disks of meropenem (10 μg), imipenem (10 μg), and ertapenem (10 μg) (Bio-Rad, France) were used. Bacterial suspensions were prepared to a density of 0.5 McFarland standard [$\approx 1 \times 10^8$ colony-forming units (CFU)/mL] and incubated at 37 °C for 18–24 hrs. *K. pneumoniae* isolates with inhibition zones for ertapenem and meropenem < 25 mm were selected for further testing.

Antibiotic susceptibility for selected strains was assessed using the automated Vitek® 2 system with AST-GN76 cards containing 12 antibiotics (piperacillin–tazobactam, ceftriaxone, ceftazidime, cefepime, ertapenem, imipenem, ciprofloxacin, levofloxacin, gentamicin, amikacin, trimethoprim–sulfamethoxazole, tigecycline) and phenotypic detection of extended-spectrum β-lactamases (ESBLs). Suspensions adjusted to 0.5 McFarland were prepared in sterile NaCl, and cards were incubated for 8–12 hrs with automatic readings every 15 min.

Colistin susceptibility was tested using the broth microdilution method (Liofilchem, Italy) according to EUCAST recommendations. Serial two-fold dilutions of colistin were prepared in cation-adjusted Mueller-Hinton broth in 96-well microtiter plates. Inocula of 0.5 McFarland suspensions were diluted to a final density of $\approx 5 \times 10^5$ CFU/mL. Plates were incubated at 37 °C for 18–20 hrs, and minimum inhibitory concentrations were recorded as the lowest concentrations showing no visible growth. Quality control was performed using *Escherichia coli* ATCC® 25922™.

Deoxyribonucleic acid isolation

Deoxyribonucleic acid (DNA) was extracted using the boiling method. Colonies from agar plates were transferred into Luria-Bertani broth and incubated overnight. A 1.5 mL aliquot was centrifuged at 12,000 revolutions *per* minute (rpm) for 2 min. The pellet was resuspended in 300 μL sterile distilled water, boiled for 10 min, cooled at -20 °C for 10 min, and centrifuged again at 12,000 rpm for 2 min. The supernatant containing DNA was transferred into a new tube and used for polymerase chain reaction (PCR) or stored at -20 °C.

Detection of carbapenemase genes by polymerase chain reaction

Carbapenemase genes (bla_{NDM} [19], bla_{KPC} [20], $bla_{OXA\text{-}48\text{-}like}$ [21], bla_{VIM} [22], and bla_{IMP} [22]) were detected using multiplex PCR. Primer sequences and amplicon sizes are listed in Table 1. PCR conditions included initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 45 s, annealing at 59 °C for 60 s, and extension at 72 °C for 60 s, with a final extension at 72 °C for 10 min. PCR products were analyzed by electrophoresis in 2% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light.

Table 1

Primers used for carbapenemase gene detection

Primer	Sequence 5′-3′	Amplicon size (bp)	Reference
bla_{NDM} Fw	GGGCAGTCGCTTCCAACGGT	475	17
bla_{NDM} Rw	GTAGTGCTCAGTGTCGGCAT		
bla_{KPC} Fw	ATGTCACTGTATCGCCGTCT	893	18
bla_{KPC} Rw	TTTTCAGAGCCTTACTGCCC		
$bla_{OXA\text{-}48\text{-}like}$ Fw	TTGGTGGCATCGATTATCGG	744	19
$bla_{OXA\text{-}48\text{-}like}$ Rw	GAGCACTTCTTTTGTGATGGC		
bla_{VIM} Fw	GATGGTGTTTGGTCGCATA	390	20
bla_{VIM} Rw	CGAATGCGCAGCACCAG		
bla_{IMP} Fw	GGAATAGAGTGGCTTAATTCTC	188	20
bla_{IMP} Rw	CCAAACCACTACGTTATCT		

NDM – New Delhi metallo-β-lactamase; KPC – *Klebsiella pneumoniae* carbapenemase; OXA-48 – oxacillinase-48; VIM – Verona integron-encoded metallo-β-lactamase; IMP – imipenemase; Fw – forward primer; Rw – reverse primer; bp – base pairs.

Statistical analysis

Baseline differences in demographic and clinical characteristics were assessed across carbapenemase genes ($bla_{OXA-48-like}$, bla_{NDM}, bla_{KPC}, and bla_{NDM} + $bla_{OXA-48-like}$). Categorical variables were summarized as counts and percentages, and continuous variables as mean ± standard deviation (SD) or median (interquartile range), as appropriate. Differences in categorical variables were tested using Fisher's exact test with Monte Carlo simulation, while age differences were assessed using one-way analysis of variance. Two-sided *p*-values < 0.05 were considered statistically significant. The primary outcome was all-cause 30-day mortality following *K. pneumoniae* isolation. Survival was analyzed using Kaplan-Meier estimates stratified by carbapenemase genes, with group differences assessed using the log-rank test. Univariable Cox proportional hazards models were constructed for candidate predictors: age, sex, study year, infection type, surgery within 30 days, ICU admission, primary diagnosis, McCabe classification, invasive devices (drain, CVC, MV, UC), and carbapenemase genes. Since age did not satisfy the linearity assumption in the log-hazard, it was categorized as a binary variable (< 70 vs. ≥ 70 years), with the cut-off chosen based on the cohort median and clinical plausibility. Predictors with $p < 0.05$ in univariable analysis or considered clinically relevant were included in the multivariable model. Variables with fewer than 10 outcome events were excluded to avoid unstable estimates. McCabe classification was omitted due to the complete separation of outcomes. The proportional hazards assumption was evaluated graphically using log-minus-log plots. Model fit was assessed using likelihood ratio tests and -2 log-likelihood statistics. Analyses were conducted using IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY, USA).

Results

Characteristics of the studied cohort

A total of 121 patients with *K. pneumoniae* HAI were included in the study, with the majority (80.2%) detected in 2023 and 19.8% in 2022 (Table 2). The patients included 75 (62.0%) males and 46 (38.0%) females with a mean ± SD of 65.3 ± 17.3 years. Clinical characteristics of the patients are presented in Table 2. A mortality within 30 days occurred in 59.5% of patients.

Table 2

Distribution of carbapenemase gene types according to patient clinical characteristics

Parameter	All isolates (n = 121)	Most frequent carbapenemase genotypes (n = 110)	$bla_{OXA-48-like}$ (n = 68)	bla_{NDM} (n = 9)	bla_{KPC} (n = 21)	bla_{NDM} + $bla_{OXA-48-like}$ (n = 12)	*p*-value*
Study year							
2022	24 (19.8)	22 (20.0)	10 (45.5)	5 (22.7)	0 (0)	7 (31.8)	**< 0.001**
2023	97 (80.2)	88 (80.0)	58 (65.9)	4 (4.5)	21 (23.9)	5 (5.7)	
Age, years	65.3 (17.3)	65.0 (17.2)	64.5 (19.2)	74.7 (7.5)	67.1 (15.0)	57.2 (17.3)	0.148§
Gender							
male	75 (62.0)	69 (62.7)	42 (60.9)	6 (8.7)	11 (15.9)	10 (14.5)	0.374
female	46 (38.0)	41 (37.2)	26 (63.4)	3 (7.3)	10 (24.4)	2 (4.9)	
Previous stay in another hospital	28 (23.1)	26 (23.6)	17 (65.4)	1 (3.8)	5 (19.2)	3 (11.5)	0.909
Type of infection**							
pneumonia	35 (28.9)	33 (31.8)	19 (57.6)	3 (9.1)	9 (27.3)	2 (6.1)	0.421
BSI	49 (40.5)	45 (40.9)	29 (64.4)	2 (4.4)	10 (22.2)	4 (8.9)	0.579
UTIs	10 (8.3)	10 (9.1)	5 (50.0)	2 (20.0)	2 (20.0)	1 (10.0)	0.421
SSIs	13 (10.7)	11 (10.0)	5 (45.5)	2 (18.2)	2 (18.2)	2 (18.2)	0.275
Comorbidities							
diabetes mellitus	25 (20.7)	22 (20.0)	8 (36.4)	5 (22.7)	7 (31.8)	2 (9.1)	**0.007**
neoplasm	22 (18.2)	22 (20.0)	15 (68.2)	1 (4.5)	6 (27.3)	0 (0)	0.197
McCabe classification							
non-fatal disease	21 (17.4)	19 (17.3)	8 (42.1)	0 (0)	4 (21.1)	7 (36.8)	
ultimately fatal disease	26 (21.5)	23 (20.9)	14 (60.9)	4 (17.4)	5 (21.7)	0 (0)	**0.007**
rapidly fatal disease	74 (61.2)	68 (61.8)	46 (67.6)	5 (7.4)	12 (17.6)	5 (5.4)	
Presence of invasive devices***							
drain	55 (45.5)	50 (45.5)	33 (66.0)	1 (2.0)	11 (22.0)	5 (10.0)	0.170
central venous catheter	101 (83.5)	94 (85.4)	60 (63.8)	5 (5.3)	19 (20.2)	10 (10.6)	0.092
mechanical ventilation	101 (83.5)	93 (84.5)	61 (65.6)	4 (4.3)	20 (21.5)	8 (8.6)	**0.002**
urinary catheter	119 (98.3)	108 (98.2)	68 (63.0)	8 (7.4)	21 (19.4)	11 (10.2)	**0.035**
Pre-infection length of stay, day							
< 14	60 (49.6)	52 (47.3)	31 (59.6)	4 (7.7)	12 (23.1)	5 (9.6)	0.802
≥ 14	61 (50.4)	58 (52.7)	37 (63.8)	5 (8.6)	9 (15.5)	7 (12.1)	
Fatal outcome within 30 days	72 (59.5)	66 (60.0)	46 (69.7)	5 (7.6)	12 (18.2)	3 (4.5)	**0.048**
Surgery within 30 days	67 (55.4)	62 (56.4)	40 (64.5)	3 (4.8)	12 (19.4)	7 (11.3)	0.557

Table 2 (continued)

Parameter	All isolates (n = 121)	Most frequent carbapenemase genotypes (n = 110)	*bla*$_{OXA-48-like}$ (n = 68)	*bla*$_{NDM}$ (n = 9)	*bla*$_{KPC}$ (n = 21)	*bla*$_{NDM}$ + *bla*$_{OXA-48-like}$ (n = 12)	*p*-value*
Type of care							
non-ICU	12 (9.9)	11 (10.0)	7 (63.6)	2 (18.2)	1 (9.1)	1 (9.1)	0.471
ICU admission	109 (90.1)	99 (90.0)	61 (61.6)	7 (7.1)	20 (20.2)	11 (11.1)	
Primary diagnosis							
cardiovascular diseases	24 (19.8)	21 (19.1)	8 (38.1)	5 (23.8)	4 (19.0)	4 (19.0)	**0.008**
gastrointestinal diseases	15 (12.4)	15 (13.6)	11 (73.3)	0 (0)	2 (13.3)	2 (13.3)	0.686
neurological diseases	11 (9.1)	10 (9.1)	8 (80.0)	0 (0)	1 (10.0)	1 (10.0)	0.845
respiratory diseases	18 (14.9)	15 (13.6)	9 (60.0)	1 (6.7)	3 (20.0)	2 (13.3)	0.936
cancer	22 (18.2)	22 (20.0)	15 (68.2)	1 (4.5)	6 (27.3)	0 (0)	0.194
injuries and intoxications	22 (18.2)	20 (18.2)	12 (60.0)	1 (5.0)	5 (25.0)	2 (10.0)	0.906
other	9 (7.4)	7 (6.4)	5 (71.4)	1 (14.3)	0 (0)	1 (14.3)	0.433

BSI – bloodstream infection; UTI – urinary tract infection; SSI – surgical site infection; ICU – intensive care unit; n – number. For other abbreviations, see Table 1.
All values are given as numbers (percentages), except for age parameter, which is expressed as mean (standard deviation).
§*p*-value for one-way analysis of variance; other *p*-values derived from Fisher's exact test (Monte Carlo). Values that differ significantly ($p < 0.05$) are bolded.
***Note:* Percentages in the first column are based on all isolates (n = 121). *Comparative analyses and corresponding percentages refer to the four most prevalent carbapenemase groups only (n = 110). ** Some patients had multiple sites of infection. *** Some patients could have several invasive devices.**

Table 3

Antimicrobial susceptibility testing results of *Klebsiella pneumoniae*

Antimicrobial	Number of isolates tested	Susceptible	Susceptible, increased exposure	Resistant
Ceftriaxone/cefotaxime	121	0 (0)	0 (0)	121 (100)
Cefepime	121	1 (0.8)	0 (0)	120 (99.2)
Gentamicin	121	16 (13.2)	0 (0)	105 (86.8)
Amikacin	121	7 (5.8)	0 (0)	114 (94.2)
Ciprofloxacin	121	1 (0.8)	0 (0)	120 (99.2)
Levofloxacin	117	2 (1.7)	0 (0)	115 (95.0)
Imipenem	121	15 (12.4)	7 (5.8)	99 (81.8)
Meropenem	121	5 (4.1)	0 (0)	116 (95.9)
Trimethoprim-sulfamethoxazole	121	1 (0.8)	0 (0)	120 (99.2)
Colistin	119	47 (38.8)	0 (0)	72 (59.5)

All values are given as numbers (percentages).
***Note*: No isolates exhibit single resistance.**

Isolates were most often obtained from blood cultures (44.6%) and bronchial aspirates (38.8%), with smaller percentages from bronchoalveolar lavage (6.6%), urine (3.3%), wound swabs (2.5%), drains (1.7%), cerebrospinal fluid (1.7%), and brain abscess (0.8%).

Antimicrobial susceptibility testing

Antimicrobial susceptibility results are summarized in Table 3. A pronounced level of multidrug resistance was detected. Dual resistance to aminoglycosides (gentamicin and amikacin) was identified in 84.3% of isolates, when 15.7% remained susceptible to both agents. High resistance rates were also observed for fluoroquinolones, with 94.2% of isolates resistant to ciprofloxacin and levofloxacin, noting that levofloxacin testing was not performed on all isolates. Resistance to carbapenems was confirmed in 95.9% of isolates, with 18.2% interpreted as susceptible, including those classified as susceptible, and increased exposure according to EUCAST criteria. According to our study definition, *K. pneumoniae* isolates were considered carbapenem-resistant if they exhibited resistance to at least one of the two tested carbapenems (imipenem or meropenem). When resistance patterns were analyzed across four major antibiotic groups, including third-generation cephalosporins, aminoglycosides, fluoroquinolones, and carbapenems, 67.8% of isolates exhibited resistance to all, highlighting the predominance of multidrug-resistant strains, while 32.2% retained at least partial susceptibility.

Distribution of carbapenemase genes during the study period

Of the 121 carbapenemase-producing isolates, 110 belonged to the four most frequent carbapenemase groups (*bla*$_{OXA-48-like}$, *bla*$_{NDM}$, *bla*$_{KPC}$, and *bla*$_{NDM}$ + *bla*$_{OXA-48-like}$), and were included in the comparative analysis. The distribution

of carbapenemase genes differed significantly between the two study years ($p < 0.001$) (Table 2).

The $bla_{OXA-48-like}$ gene was the most prevalent as the single carbapenemase gene detected in both years, with 45.5% of isolates in 2022 and 65.9% in 2023, followed by bla_{NDM}, with 22.7% in 2022 and 4.5% in 2023, while bla_{KPC} as a single carbapenemase gene was detected only in isolates from 2023 (23.9%). The most frequently detected combination of carbapenemase genes was bla_{NDM} + $bla_{OXA-48-like}$, with 31.8% in 2022 and 5.7% in 2023.

The remaining 11 isolates were excluded from the comparative analysis due to their small number and heterogeneity. Among these, the bla_{VIM} gene was detected only in combination with other carbapenemase genes, while the bla_{IMP} gene was not detected. Combinations $bla_{OXA-48-like}$ + bla_{KPC} and $bla_{OXA-48-like}$ + bla_{VIM} were each identified in two isolates in 2023. Single isolates carried bla_{NDM} + bla_{KPC} in 2022, and bla_{NDM} + bla_{VIM} and bla_{KPC} + bla_{VIM} in 2023. One isolate from 2022 harbored three carbapenemase genes (bla_{NDM} + $bla_{OXA-48-like}$ + bla_{KPC}). In addition, three isolates from 2023 did not carry any carbapenemase gene (Figure 1).

Association between carbapenemase genes and clinical characteristics of the patients

The association between carbapenemase genes and clinical characteristics of the patients is presented in Table 2. A statistically significant association was observed between carbapenemase gene distribution and diabetes mellitus status of the patients ($p = 0.007$). Non-diabetic patients were predominantly infected with *K. pneumoniae* carrying $bla_{OXA-48-like}$ (68.2%), whereas this carbapenemase was detected in 36.4% of isolates from diabetic patients. In contrast, diabetic patients were more frequently infected with *K. pneumoniae* carrying bla_{KPC} (31.8%) and bla_{NDM} (22.7%) carbapenemases.

In addition, a statistically significant association was observed between carbapenemase gene distribution and McCabe categories ($p = 0.007$) (Figure 2). In all categories, $bla_{OXA-48-like}$ was dominant (rapidly fatal disease 67.6%, ultimately fatal disease 60.9%, and non-fatal disease 42.1%); however, the relative contribution of other carbapenemase genes varied, and bla_{NDM} genes were not detected in the non-fatal disease category.

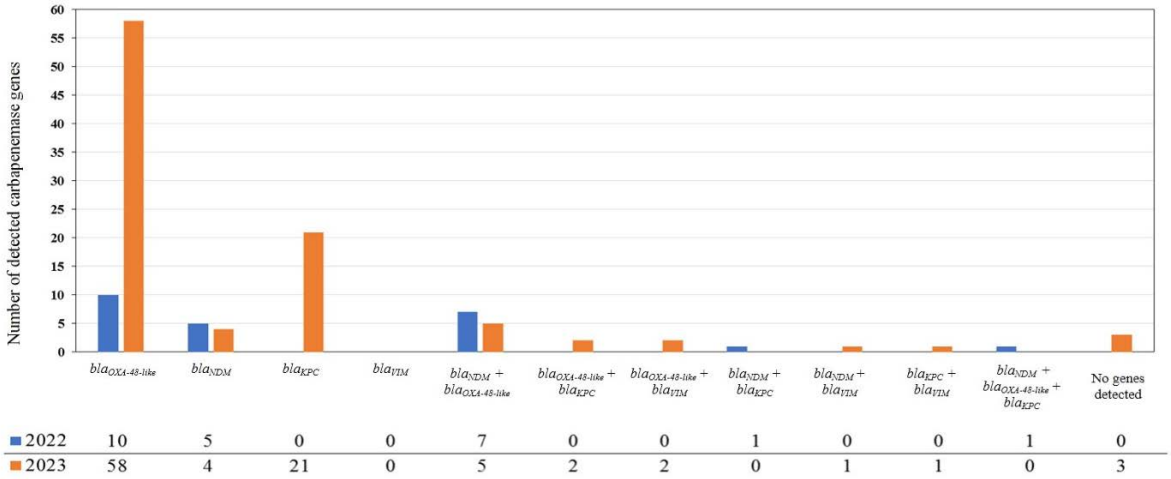

Fig. 1 – Distribution of carbapenemase genes during the study period. For abbreviations, see Table 1.

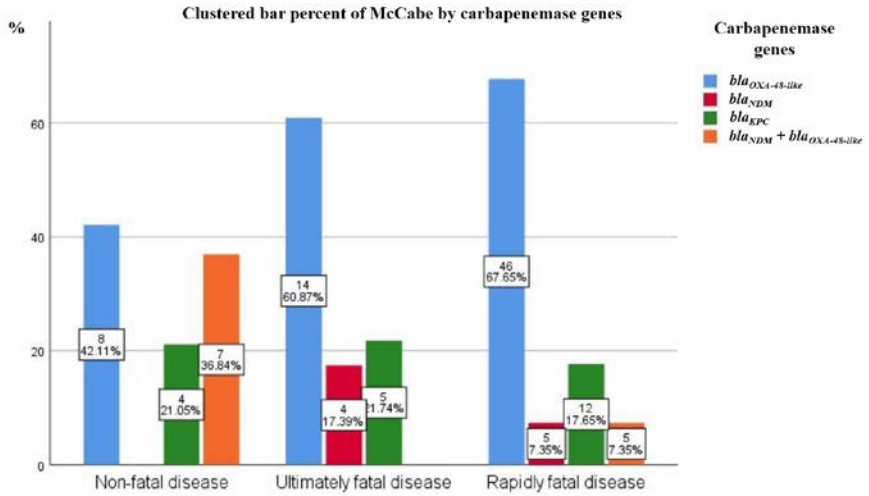

Fig. 2 – Distribution of carbapenemase genes according to McCabe classification at admission. For abbreviations, see Table 1.

Among patients on MV, the most common isolates were $bla_{OXA\text{-}48\text{-}like}$ (65.6%) and bla_{KPC} (21.5%), while bla_{NDM} was the least frequent (4.3%) compared with patients who were not on MV (p = 0.002). Urinary catheterization was nearly universal (98.3%), but the two patients without a UC carried bla_{NDM} and bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$ (p = 0.035).

Overall, 59.5% of patients died within 30 days. Among non-survivors, $bla_{OXA\text{-}48\text{-}like}$ was most frequent (69.7%), whereas among survivors, $bla_{OXA\text{-}48\text{-}like}$ was isolated in 50.0% of patients. Among non-survivors, $bla_{OXA\text{-}48\text{-}like}$ was most frequent (69.7%), following bla_{KPC} (18.2%), bla_{NDM} (7.6%), and bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$ (4.5%) (p = 0.048).

Kaplan-Meier analysis of 30-day survival

Kaplan-Meier survival analysis was performed to evaluate 30-day survival after *K. pneumoniae* isolation according to carbapenemase gene type (Figure 3). The overall median survival time was 13 days [95% confidence interval (CI): 8.7–17.3], while the mean survival was 16.1 days (95% CI: 13.8–18.4). In the subgroup analysis by carbapenemase gene type, the median survival was 12 days (95% CI: 7.5–16.5) for $bla_{OXA\text{-}48\text{-}like}$, 14 days (95% CI: 0–40.3) for bla_{NDM}, and 11 days (95% CI: 0–33.4) for bla_{KPC} producers. Median survival could not be estimated for the bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$ group due to censoring, although the mean survival was longest in this group (22.8 days, 95% CI: 15.8–29.9) (Table 4).

The log-rank test showed no statistically significant difference in 30-day survival distributions across carbapenemase groups (χ^2 = 4.83, df = 3, p = 0.185), although isolates carrying the $bla_{OXA\text{-}48\text{-}like}$ gene demonstrated a clinically notable trend toward lower survival probabilities.

Cox regression analysis of predictors of 30-day survival

Results of univariable Cox regression analysis are presented in Table 5. In univariable Cox regression, age ≥ 70 years was associated with an increased risk of 30-day mortality [hazard ratio (HR): 1.96; 95% CI: 1.21–3.15; p = 0.006]. Among infection types, BSI was linked to a higher hazard of death (HR: 1.75; 95% CI: 1.01–2.78; p = 0.018), whereas pneumonia was associated with a lower risk (HR: 0.52; 95% CI: 0.29–0.91; p = 0.024). ICU admission (HR: 3.60; 95% CI: 1.13–11.46; p = 0.030), recent surgery within 30 days (HR: 1.82; 95% CI: 1.14–2.90; p = 0.013), and respiratory diseases as the primary diagnosis (HR: 1.80; 95% CI: 1.01–3.23; p = 0.049) were also significant predictors. In contrast, injuries and intoxications as the primary diagnosis appeared protective (HR: 0.45; 95% CI: 0.21–0.95; p = 0.036). Notably, predictors such as BSI and respiratory diseases had lower confidence-interval bounds close to 1.00 (1.01 and 1.01, respectively), indicating statistically fragile yet clinically plausible associations.

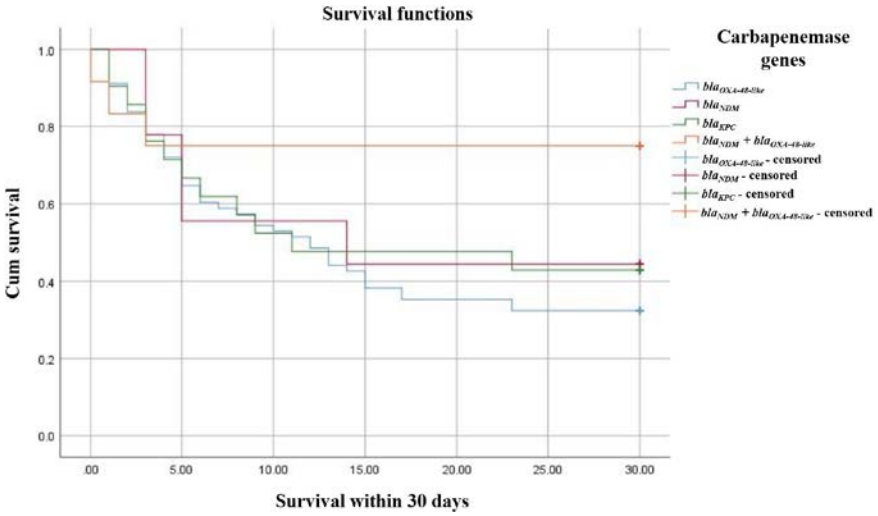

Fig. 3 – Kaplan-Meier survival estimates within 30 days after *Klebsiella pneumoniae* isolation according to carbapenemase type.
Cum – cumulative. For other abbreviations, see Table 1.
***Note*: The log-rank test showed no statistically significant difference in 30-day survival distributions across carbapenemase groups (χ^2 = 4.83, df = 3, p = 0.185).**

Table 4

Kaplan-Meier survival estimates within 30 days after *Klebsiella pneumoniae* isolation

Carbapenemase genes	Median survival (days)	95% CI for median	Mean survival (days)	95% CI for mean
$bla_{OXA\text{-}48\text{-}like}$	12	7.5–16.5	14.8	12.0–17.5
bla_{NDM}	14	0–40.3	16.7	8.6–24.7
bla_{KPC}	11	0–33.4	16.5	11.1–21.8
bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$*	–	–	22.8	15.8–29.9
Overall	13	8.7–17.3	16.1	13.8–18.4

CI – confidence interval. For other abbreviations, see Table 1.
***Note*: *Median not estimable as > 50% of patients were censored at 30 days.**

Table 5

Univariable Cox regression analysis of factors associated with 30-day mortality

Variable	Survivors (n = 49)	Non-survivors (n = 72)	HR (95% CI)	*p*-value
Study year				
2022	14 (28.6)	10 (13.9)	Ref –	–
2023	35 (71.4)	62 (86.1)	1.79 (0.91–3.49)	0.088
Age, years	57.8 ± 18.2	70.4 ± 14.7		
< 70	33 (67.3)	28 (38.9)	Ref –	–
≥ 70	16 (32.7)	44 (61.1)	1.96 (1.21–3.15)	0.006
Gender				
female	16 (32.7)	30 (41.7)	Ref –	–
male	33 (67.3)	42 (58.3)	0.74 (0.46–1.18)	0.211
Previous hospital stay	10 (20.4)	18 (25)	1.05 (0.61–1.79)	0.855
Type of infection				
pneumonia	20 (40.8)	15 (20.8)	0.52 (0.29–0.91)	0.024
BSI	15 (30.6)	34 (47.2)	1.75 (1.01–2.78)	0.018
UTI	5 (10.2)	5 (6.9)	0.82 (0.32–2.03)	0.664
SSI	8 (16.3)	5 (6.9)	0.54 (0.22–1.35)	0.191
Comorbidities				
diabetes mellitus	9 (18.4)	16 (22.2)	1.11 (0.64–1.94)	0.700
neoplasm	8 (16.3)	14 (19.4)	1.08 (0.60–1.94)	0.785
McCabe classification				
non-fatal	21 (42.9)	0 (0)	– –	–
ultimately fatal	15 (30.6)	11 (15.3)	– –	–
rapidly fatal	13 (26.5)	61 (84.7)	– –	–
Invasive devices				
drain	26 (53.1)	29 (40.3)	0.77 (0.48–1.23)	0.273
central venous catheter	43 (87.8)	58 (80.6)	0.88 (0.49–1.59)	0.683
mechanical ventilation	40 (81.6)	61 (84.7)	1.25 (0.66–2.38)	0.489
urinary catheter	47 (95.9)	72 (100)	1.82 (0.25–13.1)	0.553
Other predictors				
surgery within 30 days	35 (71.4)	32 (44.4)	1.82 (1.14–2.90)	0.013
pre-infection LOS ≥ 14 days	29 (59.2)	32 (44.4)	0.75 (0.47–1.19)	0.221
ICU admission	40 (81.6)	69 (95.8)	3.60 (1.13–11.46)	0.030
Primary diagnosis				
cardiovascular	9 (18.4)	15 (20.8)	0.97 (0.55–1.72)	0.927
gastrointestinal	5 (10.2)	10 (13.9)	1.52 (0.77–2.96)	0.223
neurological	6 (12.2)	5 (6.9)	0.60 (0.24–1.50)	0.276
respiratory	4 (8.2)	14 (19.4)	1.80 (1.01–3.23)	0.049
cancer	8 (16.3)	14 (19.4)	1.08 (0.60–1.94)	0.785
injuries/intoxications	14 (28.6)	6 (8.3)	0.45 (0.21–0.95)	0.036
other	3 (6.1)	6 (8.3)	1.30 (0.56–3.00)	0.537
Carbapenemase genes				
$bla_{OXA\text{-}48\text{-}like}$	22 (44.9)	46 (63.9)	1.50 (0.92–2.43)	0.097
bla_{NDM}	4 (8.2)	5 (6.9)	0.91 (0.36–2.24)	0.829
bla_{KPC}	9 (18.4)	12 (16.7)	0.96 (0.51–1.79)	0.903
bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$	9 (18.4)	3 (4.2)	0.33 (0.10–1.06)	0.063

LOS – length of stay; HR – hazard ratio; Ref – reference category.
For other abbreviations, see Tables 1, 2, and 4.
All values are given as numbers (percentages) or mean ± standard deviation.

Several variables did not reach statistical significance but showed HRs and CIs suggestive of potential clinical relevance. The presence of the $bla_{OXA\text{-}48\text{-}like}$ gene was associated with an elevated mortality risk (HR: 1.50; 95% CI: 0.92–2.43; $p = 0.097$), with the CI narrowly crossing 1.00.

McCabe classification showed complete HRs prediction of outcome: no deaths occurred among patients with non-fatal disease, whereas nearly all patients with rapidly fatal disease died within 30 days. Because of this complete separation, HR could not be reliably estimated for McCabe categories in univariable Cox regression, although descriptive analysis clearly demonstrated its strong prognostic value. For this reason, McCabe's classification was excluded from the multivariable analysis.

In the multivariable Cox regression model, age ≥ 70 years, BSI, and ICU admission remained independent predictors of 30-day mortality (Table 6). Patients aged ≥ 70 had nearly a two-fold increased hazard of death compared with those younger than 70 (HR: 1.98; 95% CI: 1.23–3.20; $p = 0.005$). BSI was associated with a 63% higher hazard (HR: 1.63; 95% CI: 1.02–2.61; $p = 0.040$), while ICU admission was associated with more than a three-fold increased hazard (HR: 3.35; 95% CI: 1.05–10.72; $p = 0.041$). Sex was not significantly associated with survival in this model.

Table 6

Univariable and multivariable Cox regression analysis of predictors of 30-day mortality

Variable	Univariable HR (95% CI)	*p*-value	Multivariable HR (95% CI)	*p*-value
Age ≥ 70 years	1.96 (1.21–3.15)	0.006	1.98 (1.23–3.20)	0.005
BSI	1.75 (1.01–2.78)	0.018	1.63 (1.02–2.61)	0.040
ICU admission	3.60 (1.13–11.46)	0.030	3.35 (1.05–10.72)	0.041
Pneumonia	0.52 (0.29–0.91)	0.024	–	n.s.
Surgery < 30 days	1.82 (1.14–2.90)	0.013	–	n.s.
Respiratory diagnosis	1.80 (1.01–3.23)	0.049	–	n.s.
Injuries/intoxications	0.45 (0.21–0.95)	0.036	–	n.s.

n.s. – not significant. For other abbreviations, see Tables 2, 4, and 5.

***Note*: Variables considered in the stepwise Cox regression were age ≥ 70 years, sex, BSI, ICU admission, pneumonia, recent surgery, and respiratory diagnosis. Only significant predictors are presented.**

Discussion

In this single-center cohort of hospitalized patients with HAI *K. pneumoniae* infections, 30-day all-cause mortality was high (59.5%). Independent predictors of poor outcome included age ≥ 70 years, BSI, and ICU admission, while carbapenemase genes did not independently influence survival. These findings emphasize that host and clinical severity remain the dominant determinants of short-term outcomes.

In our study, the mortality rate exceeds that reported in most multicenter European cohorts. It is important to differentiate healthcare between different countries. In the Serbian context, available data and literature indicate that death most often occurs in hospitals rather than at home. During the coronavirus disease 2019 pandemic, as many as 94.3% of deaths occurred in hospitals and only 5.7% at home [23]. Furthermore, the dying process has, in most cases, shifted from homes to healthcare facilities, highlighting that palliative care is still not fully integrated into everyday practice in Serbia [24].

However, Isler et al. [25] recently reported 30-day mortality of 44% in carbapenemase-harboring carbapenem-resistant *Klebsiella* spp. BSI infections, while a systematic review and meta-analysis indicated that more than 50% of ICU, HAI, CRKP, and ESBL-producing *K. pneumoniae* were associated with significantly higher 30-day mortality rates (estimated at ~ 29%) [26].

Similarly, Maraolo et al. [27] demonstrated an adjusted two-fold increase in death with CRKP vs. carbapenem-sensitive *K. pneumoniae* isolates. By contrast, northern European series, such as Fostervold et al. [28], documented substantially lower case-fatality (~12%), likely reflecting lower prevalence of resistance and more favorable baseline patient status. Our findings, therefore, align with the higher end of published mortality estimates, reflecting the severe case mix and therapeutic limitations in our setting.

Our results can also be compared with those of Soares de Moraes et al. [11], who investigated 107 patients with *K. pneumoniae* BSI in Brazil, of whom 50.5% carried carbapenem-resistant isolates. In their cohort, bla_{KPC} was the dominant carbapenemase, whereas in our study, $bla_{OXA\text{-}48\text{-}like}$ predominated, followed by bla_{KPC} and bla_{NDM}. Mortality was elevated in both studies, but different prognostic factors emerged. Soares de Moraes et al. [11] identified renal failure, liver failure, and extensively/pandrug-resistant status as predictors of death, while in our analysis, age ≥ 70 years, BSI, and ICU admission were most strongly associated with 30-day mortality. These results highlight that although resistance mechanisms differ across regions, mortality remains high and is largely determined by host condition and severity of illness rather than the presence of the carbapenemase genes alone.

Comparable mortality rates have been reported in several other high-risk cohorts. In Brazil, Andrey et al. [12] observed a 30-day mortality rate of 60% in patients with KPC-producing *K. pneumoniae* BSIs, with the sequence type 16 clone reaching nearly universal fatality. In Italy, Falcone et al. [29] reported a 30-day mortality rate of 40% in ICU patients with septic shock due to KPC-producing *K. pneumonia*. They demonstrated that a colistin-containing regimen, the use of two or more *in vitro* active antibiotics as definite therapy, and control of a removable source of infection were independently associated with a favorable outcome, while infection due to a colistin-resistant strain and an intra-abdominal source of infection were independently associated with death. Giacobbe et al. [30] further demonstrated that in the subgroup with colistin-resistant isolates, mortality reached ~ 50%, underscoring the impact of last-line resistance. Taken together, these findings confirm that our observed mortality lies at the upper range of global experience and is consistent with cohorts where advanced age, critical illness, and therapeutic limitations converge to drive poor outcomes.

Although in our cohort, the presence of the carbapenemase genes was not significantly associated with short-term survival, genetic information remains clinically relevant for antimicrobial selection. In particular, data from the Hellenic Ceftazidime/Avibactam Registry demonstrated markedly better outcomes among patients infected with KPC-producing *K. pneumoniae* compared with those harboring NDM or OXA-48 enzymes, underscoring the therapeutic importance of precise carbapenemase identification [31].

We observed high resistance rates to most of the tested antibiotics. Resistance to ceftriaxone/cefotaxime was universal (100%), far exceeding the European Union/European Economic Area (EU/EEA) population-weighted average of 34.8% reported by ECDC for 2023 [8]. Similarly, resistance to cefepime reached 99.2%, underscoring the diminished therapeutic value of extended-spectrum cephalosporins in our setting.

Very high resistance rates were also recorded for aminoglycosides, with 86.8% of isolates resistant to gentamicin and 94.2% to amikacin, compared to the European average of 23.6% [8]. Comparable patterns were observed for fluoroquinolones, with resistance rates of 99.2% for ciprofloxacin and 95.0% for levofloxacin; however, susceptibility testing was not performed for four isolates. By contrast, the European average was 33.7% [8]. These findings suggest that aminoglycosides and fluoroquinolones have lost nearly all clinical utility in our cohort, unlike in the broader European context.

Another major concern is the extremely high resistance to carbapenems, which have traditionally been considered last-line agents. Resistance to imipenem was observed in 81.8% of isolates, while resistance to meropenem reached 95.9%, compared with the EU/EEA average of 13.3% [8]. Although ECDC data indicated a gradual increase in carbapenem resistance over the 2019–2023 period, the levels observed in our study are considerably higher and align with reports of heavier resistance burdens in southern and eastern Europe [8].

High resistance was also observed for trimethoprim-sulfamethoxazole (99.2%).

Colistin showed variable results, with 59.5% of isolates being resistant and 38.8% remaining susceptible. Results are based on 119 isolates, as testing was unavailable for two isolates. This finding is clinically relevant given its role as a last-resort therapeutic option for carbapenem-resistant infections.

Dual resistance to both aminoglycosides was present in 84.3% of isolates, while 94.2% were resistant to both fluoroquinolones. Simultaneous resistance to both carbapenems was detected in 81.8% of isolates. In comparison, ECDC reported that 21.0% of *K. pneumoniae* isolates across the EU/EEA showed combined resistance to third-generation cephalosporins, fluoroquinolones, and aminoglycosides, while 11.1% showed combined resistance to four groups of antibiotics, including carbapenems [8]. In our study, 67.8% of isolates were simultaneously resistant to all four groups, indicating a predominance of MDR *K. pneumoniae* strains. Similar trends were also reported in the Niš region (southern Serbia) during the 2014–2018 period, where *Klebsiella* spp. MDR isolates showed a decreasing trend in susceptibility to cephalosporins and fluoroquinolones, with resistance rates to ciprofloxacin and levofloxacin consistently above 60% [32].

The exceptionally high resistance rates observed in our study should be interpreted within the clinical context of a tertiary-care referral institution. These patients typically presented with severe or advanced clinical conditions, with nearly half diagnosed with BSI.

The majority required ICU management (90.1%) with exposure to invasive devices such as UC (98.3%), MV (83.5%), and CVC (83.5%), all of which are recognized risk factors for colonization and infection with MDR organisms. Extensive prior antimicrobial exposure combined with critical illness and invasive support creates strong selective pressure favoring the emergence and persistence of resistant strains. Therefore, the predominance of MDR *K. pneumoniae* isolates in our cohort is not only expected but also reflects the therapeutic challenges faced in high-risk clinical environments.

In the past period, there has been a rapid dissemination of MDR strains, including carbapenem-resistant strains [6–8]. A rapid and extensive dissemination of CRKP strains is caused by horizontal genetic transfer, since carbapenemase genes are mainly plasmid encoded [2].

During the study period, we detected $bla_{OXA\text{-}48\text{-}like}$ as the most dominant carbapenemase gene, followed by bla_{KPC}, bla_{NDM}, and bla_{VIM}. Moreover, the most common co-producers were bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$.

The distribution of carbapenemase genes in our study is consistent with previous reports from our country [33–35]. In the study by Zornic et al. [33], the majority of isolates carried $bla_{OXA\text{-}48\text{-}like}$, followed by bla_{KPC} and bla_{NDM} genes, while the majority of co-producers harbored $bla_{OXA\text{-}48\text{-}like}$ and bla_{NDM} genes. Additionally, bla_{NDM} + bla_{VIM} was detected in one isolate. In the other two studies, the most prevalent carbapenemase gene was $bla_{OXA\text{-}48\text{-}like}$, which was associated with the high-risk sequence type 101 clone. The plasmid that carried the $bla_{OXA\text{-}48\text{-}like}$ gene also carried the ESBL encoding gene $bla_{CTX\text{-}M\text{-}15}$ and several other resistance genes [34, 35]. In contrast to our results, a 2017 study of MDR *K. pneumoniae* strains reported bla_{NDM} was the most prevalent gene [36].

Our results are also related to the regional carbapenemase gene distribution, showing similar resistance profiles of *K. pneumoniae* strains with the predominant $bla_{OXA\text{-}48\text{-}like}$ carbapenemase circulating in neighboring countries. The predominance of $bla_{OXA\text{-}48\text{-}like}$ observed in our study is consistent with findings from several countries in the Balkan region [37–40].

In our study, the bla_{VIM} gene was detected in only four isolates from 2023, indicating its limited circulation compared with other carbapenemase genes. This finding is consistent with data from neighboring countries, where VIM-producing *K. pneumoniae* strains have been reported sporadically or replaced over time by other carbapenemase types [41]. Together, these findings suggest that in the Balkan region, bla_{VIM} genes occur at low frequencies and are being progressively displaced by the expansion of $bla_{OXA\text{-}48\text{-}like}$ and bla_{NDM}, mirroring the trend observed in our cohort.

With respect to comorbidities, diabetes mellitus was the only condition showing a statistically significant association with carbapenemase gene carriage. Among these patients, $bla_{OXA\text{-}48\text{-}like}$ and bla_{KPC} genes predominated, followed by bla_{NDM} and the bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$ combination. A similar distribution of carbapenemase genes was observed among patients with neoplasms; however, this was not statistically significant. Given that these patients are typically immunocompromised, the presence of carbapenem-resistant isolates may further complicate treatment and increase the risk of adverse clinical outcomes. In contrast to our results, the study from Romania detected co-producers bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$ as the dominant carbapenemase genes, but without a statistically significant association with diabetes mellitus and neoplasms [13].

A statistically significant association was also observed for the McCabe classification. The majority of cases were

classified as having a rapidly fatal disease, followed by an ultimately fatal disease. Within these categories, *bla*$_{OXA-48-like}$ and *bla*$_{KPC}$ were the most frequently detected carbapenemase genes, suggesting that these enzymes may be linked to more severe infections or reflect the higher vulnerability of critically ill patients. The majority of patients were treated in the ICU, frequently requiring MV and UC, both of which were significantly associated with carbapenemase gene distribution. Although our results confirm ICU stay, but not MV, CVC, or UC as risk factors for fatal outcome, other studies emphasize the importance of invasive procedures as potential risk factors for colonization or infection by CPKP [6, 10, 11].

Kaplan-Meier analysis showed differences in survival across carbapenemase genes, with the *bla*$_{NDM}$ + *bla*$_{OXA-48-like}$ group exhibiting the longest median survival, yet no statistically significant association with 30-day survival was detected. This aligns with a recent multicenter cohort where mortality did not differ between KPC- and NDM-producing isolates after adjustment for therapy and clinical severity [15]. In contrast, other cohorts reported worse outcomes with KPC compared with NDM producers [14]. A Spanish study focusing on OXA-48 producers further suggests that appropriate therapy, rather than the enzyme class *per se*, is the main determinant of short-term survival [42].

The marked shift in carbapenemase distribution between 2022 and 2023, characterized by a decline in NDM producers and a substantial increase in OXA-48 and KPC, may reflect changes in local epidemiology, plasmid transmission dynamics, selective antimicrobial pressure, and patient case mix. Similar year-to-year variability has been reported in tertiary hospitals across the Balkan region, where OXA-48 enzymes have rapidly become dominant over time [13]. Although our study design does not allow causal inference, these trends underscore the necessity of continuous molecular surveillance.

In addition to patient-related and microbiological factors, contextual characteristics of our healthcare system likely contribute to the high burden of CRKP and the poor outcomes observed. IPC programs are formally implemented in our hospital, but adherence to hand hygiene, contact precautions, and device-related bundles is often inconsistent in daily practice. These challenges are compounded by a chronic shortage of adequately trained nursing staff, with a considerable proportion of nurses newly qualified or retrained and with limited experience in caring for critically ill patients. Such constraints may reduce compliance with complex IPC procedures and delay early recognition of clinical deterioration, thereby further increasing the risk of adverse outcomes in this vulnerable population. Future studies should explicitly evaluate the impact of these environmental and organizational factors on CRKP transmission and mortality.

This study has several limitations. First, it was conducted in a single tertiary-care center, which may limit the generalizability of the findings. Second, the retrospective design is prone to selection and information bias, and some relevant clinical variables were not consistently available. Third, the relatively small sample size reduced statistical power, resulting in wide or imprecise CIs for several predictors. In some cases, the CIs were close to 1.00, including both statistically non-significant and statistically significant associations, indicating limited precision and the possibility that certain effects may be underestimated or overestimated. These findings should therefore be interpreted with caution and validated in larger cohorts. The limited number of events also affected age modeling, where different categorizations produced similar trends but varied in stability due to sparse events within strata. Additionally, although molecular characterization of carbapenemase genes was performed, whole-genome sequencing was not available and would have enabled a more detailed examination of resistance mechanisms and transmission dynamics. Finally, treatment-related data and infection prevention factors were not analyzed in depth, limiting conclusions regarding therapeutic effectiveness and environmental contributors to patient outcomes.

The strengths of our study include the integration of clinical outcomes with both phenotypic and genotypic characterization of CRKP isolates, the setting in a large tertiary-care center that reflects real-world practice, and the contemporaneous data capturing the ongoing regional shift toward NDM and OXA-48 producers. Moreover, our study represents a foundational effort to understand *K. pneumoniae* resistance in our hospital.

Conclusion

In this retrospective cohort of patients with healthcare-associated infections caused by carbapenem-resistant *Klebsiella pneumoniae*, 30-day mortality was high. Carbapenemase genes were not independently associated with short-term survival, whereas age ≥ 70 years, bloodstream infection, and intensive care unit stay emerged as the main predictors of mortality. These findings suggest that host factors and clinical severity, rather than specific carbapenemase genes, are the key determinants of outcome in this high-risk population. Our findings contribute valuable epidemiological insights from a Balkan tertiary-care setting, highlighting the regional predominance of New Delhi metallo-β-lactamase and oxacillinase-48 producers. Genes that encode *Klebsiella pneumoniae* carbapenemase and Verona integron-encoded metallo-β-lactamase carbapenemases were less prevalent and detected only in isolates from 2023, while the *bla imipenemase* gene was not detected in our study. Taken together, these observations reinforce the importance of coordinated local, regional, and international efforts to mitigate the impact of carbapenem-resistant *Klebsiella pneumoniae* and reduce its associated mortality.

Acknowledgement

This research was funded by the Ministry of Defence of the Republic of Serbia (grant number Faculty of Medicine MMA 02/23-25).

R E F E R E N C E S

1. *Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL.* Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. Clin Microbiol Rev 2012; 25(4): 682–707. DOI: 10.1128/CMR.05035-11.
2. *Pitout JD, Nordmann P, Poirel L.* Carbapenemase-Producing Klebsiella pneumoniae, a Key Pathogen Set for Global Nosocomial Dominance. Antimicrob Agents Chemother 2015; 59(10): 5873–84. DOI: 10.1128/AAC.01019-15.
3. *Caliskan-Aydogan O, Alocilja EC.* A Review of Carbapenem Resistance in Enterobacterales and Its Detection Techniques. Microorganisms 2023; 11(6): 1491. DOI: 10.3390/microorganisms11061491.
4. *Zhou R, Fang X, Zhang J, Zheng X, Shangguan S, Chen S,* et al. Impact of carbapenem resistance on mortality in patients infected with Enterobacteriaceae: a systematic review and meta-analysis. BMJ Open 2021; 11(12): e054971. DOI: 10.1136/bmjopen-2021-054971.
5. *Sati H, Carrara E, Savoldi A, Hansen P, Garlasco J, Campagnaro E,* et al. The WHO Bacterial Priority Pathogens List 2024: a prioritisation study to guide research, development, and public health strategies against antimicrobial resistance. Lancet Infect Dis 2025; 25(9): 1033–43. DOI: 10.1016/S1473-3099(25)00118-5.
6. *Lin XC, Li CL, Zhang SY, Yang XF, Jiang M.* The Global and Regional Prevalence of Hospital-Acquired Carbapenem-Resistant Klebsiella pneumoniae Infection: A Systematic Review and Meta-analysis. Open Forum Infect Dis 2024; 11(2): ofad649. DOI: 10.1093/ofid/ofad649.
7. *European Centre for Disease Prevention and Control.* Rapid risk assessment -Carbapenem-resistant Enterobacterales: third update [Internet]. Stockholm: ECDC; 2025 [cited 2025 Dec 24]. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/risk-assessment-carbapenem-resistant-enterobacterales-third-update-february-2025_0.pdf
8. *European Centre for Disease Prevention and Control, World Health Organization.* Antimicrobial resistance surveillance in Europe 2023: 2021 data. [Internet]. LU: Publications Office of the EU; 2023 [cited 2025 Sep 24; accessed 2025 Dec 24]. Available from: https://data.europa.eu/doi/10.2900/63495
9. *Brkic S, Cirkovic I.* Carbapenem-Resistant Enterobacterales in the Western Balkans: Addressing Gaps in European AMR Surveillance Map. Antibiotics (Basel) 2024; 13(9): 895. DOI: 10.3390/antibiotics13090895.
10. *Ćirković I, Marković-Denić L, Bajčetić M, Dragovac G, Đorđević Z, Mioljević V,* et al. Microbiology of Healthcare-Associated Infections: Results of a Fourth National Point Prevalence Survey in Serbia. Antibiotics (Basel) 2022; 11(9): 1161. DOI: 10.3390/antibiotics11091161.
11. *Soares de Moraes L, Gomes Magalhaes GL, Material Soncini JG, Pelisson M, Eches Perugini MR, Vespero EC.* High mortality from carbapenem-resistant Klebsiella pneumoniae bloodstream infection. Microb Pathog 2022; 167: 105519. DOI: 10.1016/j.micpath.2022.105519.
12. *Andrey DO, Pereira Dantas P, Martins WBS, Marques De Carvalho F, Almeida LGP, Sands K,* et al. An Emerging Clone, Klebsiellapneumoniae Carbapenemase 2-Producing K. pneumoniae Sequence Type 16, Associated With High Mortality Rates in a CC258-Endemic Setting. Clin Infect Dis 2020; 71(7): e141–50. DOI: 10.1093/cid/ciz1095.
13. *Lazar DS, Nica M, Dascalu A, Oprisan C, Albu O, Codreanu DR,* et al. Carbapenem-Resistant NDM and OXA-48-like Producing K. pneumoniae: From Menacing Superbug to a Mundane Bacteria; A Retrospective Study in a Romanian Tertiary Hospital. Antibiotics (Basel) 2024; 13(5): 435. DOI: 10.3390/antibiotics13050435.
14. *Seo H, Kim HJ, Kim MJ, Chong YP, Kim SH, Lee SO,* et al. Comparison of clinical outcomes of patients infected with KPC- and NDM-producing Enterobacterales: a retrospective cohort study. Clin Microbiol Infect 2021; 27(8): 1167.e1–8. DOI: 10.1016/j.cmi.2020.09.043.
15. *Dickstein Y, Yahav D, Tiseo G, Mussini C, Franceschini E, Santoro A,* et al. Carbapenemase type and mortality in blood-stream infections caused by carbapenemase-producing enterobacterales: a multicenter retrospective cohort study. Infection 2025; 53(6): 2491–501. DOI: 10.1007/s15010-025-02584-y.
16. *Mitchell BG, Gardner A, Stone PW, Hall L, Pogorzelska-Maziarz M.* Hospital Staffing and Health Care-Associated Infections: A Systematic Review of the Literature. Jt Comm J Qual Patient Saf 2018; 44(10): 613–22. DOI: 10.1016/j.jcjq.2018.02.002.
17. *Santos-Marques C, Ferreira H, Gonçalves Pereira S.* Infection prevention and control strategies against carbapenem resistant Enterobacteriaceae - a systematic review. J Infect Prev 2022; 23(4): 167–85. DOI: 10.1177/17571774211066762.
18. *Institute of Public Health of Serbia "Dr Milan Jovanović Batut".* Healthcare-Associated Infections Definitions. Belgrade: Ministarstvo zdravlja Republike Srbije; 2022. p. 116. (Serbian)
19. *Manchanda V, Rai S, Gupta S, Rautela RS, Chopra R, Rawat DS,* et al. Development of TaqMan real-time polymerase chain reaction for the detection of the newly emerging form of carbapenem resistance gene in clinical isolates of Escherichia coli, Klebsiella pneumoniae, and Acinetobacter baumannii. Indian J Med Microbiol 2011; 29(3): 249–53. DOI: 10.4103/0255-0857.83907.
20. *Bratu S, Tolaney P, Karumudi U, Quale J, Mooty M, Nichani S,* et al. Carbapenemase-producing Klebsiella pneumoniae in Brooklyn, NY: molecular epidemiology and in vitro activity of polymyxin B and other agents. J Antimicrob Chemother 2005; 56(1): 128–32. DOI: 10.1093/jac/dki175.
21. *Poirel L, Potron A, Nordmann P.* OXA-48-like carbapenemases: the phantom menace. J Antimicrob Chemother 2012; 67(7): 1597–606. DOI: 10.1093/jac/dks121.
22. *Ellington MJ, Kistler J, Livermore DM, Woodford N.* Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. J Antimicrob Chemother 2007; 59(2): 321–2. DOI: 10.1093/jac/dkl481.
23. *Stevanović A, Šantrić-Milićević M, Todorović J, Rajović N, Rosić N, Bjelobrk G.* COVID-19 premature mortality in Serbia: Does the place of death matter? Eur J Public Health 2024; 34(Suppl 3): ckae144.1921. DOI: 10.1093/eurpub/ckae144.1921.
24. *Maric DM.* Attitudes towards death and end-of-life care. Med Istraž 2023; 56(4): 81–5. DOI: 10.5937/medi56-46957.
25. *Isler B, Özer B, Çınar G, Aslan AT, Vatansever C, Falconer C,* et al. Characteristics and outcomes of carbapenemase harbouring carbapenem-resistant Klebsiella spp. bloodstream infections: a multicentre prospective cohort study in an OXA-48 endemic setting. Eur J Clin Microbiol Infect Dis 2022; 41(5): 841–7. DOI: 10.1007/s10096-022-04425-4.
26. *Li D, Huang X, Rao H, Yu H, Long S, Li Y,* et al. Klebsiella pneumoniae bacteremia mortality: a systematic review and meta-analysis. Front Cell Infect Microbiol 2023; 13: 1157010. DOI: 10.3389/fcimb.2023.1157010.
27. *Maraolo AE, Corcione S, Grossi A, Signori A, Alicino C, Hussein K,* et al. The Impact of Carbapenem Resistance on Mortality in Patients With Klebsiella Pneumoniae Bloodstream Infection: An Individual Patient Data Meta-Analysis of 1952 Patients. Infect Dis Ther 2021; 10(1): 541–58. DOI: 10.1007/s40121-021-00408-8.
28. *Fostervold A, Raffelsberger N, Hetland MAK, Bakksjø R, Bernhoff E, Samuelsen Ø,* et al. Risk of death in Klebsiella pneumoniae bloodstream infections is associated with specific phylogenetic

lineages. J Infect 2024; 88(5): 106155. DOI: 10.1016/j.jinf.2024.106155.
29. *Falcone M, Russo A, Iacovelli A, Restuccia G, Ceccarelli G, Giordano A,* et al. Predictors of outcome in ICU patients with septic shock caused by Klebsiella pneumoniae carbapenemase-producing K. pneumoniae. Clin Microbiol Infect 2016; 22(5): 444–50. DOI: 10.1016/j.cmi.2016.01.016.
30. *Giacobbe DR, Del Bono V, Trecarichi EM, De Rosa FG, Giannella M, Bassetti M,* et al. Risk factors for bloodstream infections due to colistin-resistant KPC-producing Klebsiella pneumoniae: results from a multicenter case-control-control study. Clin Microbiol Infect 2015; 21(12): 1106.e1–8. DOI: 10.1016/j.cmi.2015.08.001.
31. *Karaiskos I, Daikos GL, Gkoufa A, Adamis G, Stefos A, Symbardi S,* et al. Ceftazidime/avibactam in the era of carbapenemase-producing Klebsiella pneumoniae: experience from a national registry study. J Antimicrob Chemother 2021; 76(3): 775–83. DOI: 10.1093/jac/dkaa503.
32. *Veličković-Radovanović R, Stefanović N, Damnjanović I, Kocić B, Mladenović-Antić S, Dinić M,* et al. Antibiotic utility and susceptibility changes of multidrug-resistant Escherichia coli and Klebsiella spp: 5-year experience in a tertiary healthcare centre. Eur J Hosp Pharm 2022; 29(2): 84–9. DOI: 10.1136/ejhpharm-2021-002758.
33. *Zornic S, Petrovic I, Lukovic B.* In vitro activity of imipenem/relebactam and ceftazidime/avibactam against carbapenem-resistant Klebsiella pneumoniae from blood cultures in a University hospital in Serbia. Acta Microbiol Immunol Hung 2023; 70(3): 187–92. DOI: 10.1556/030.2023.02108.
34. *Palmieri M, D'Andrea MM, Pelegrin AC, Mirande C, Brkic S, Cirkovic I,* et al. Genomic Epidemiology of Carbapenem- and Colistin-Resistant Klebsiella pneumoniae Isolates From Serbia: Predominance of ST101 Strains Carrying a Novel OXA-48 Plasmid. Front Microbiol 2020; 11: 294. DOI: 10.3389/fmicb.2020.00294.
35. *Novović K, Trudić A, Brkić S, Vasiljević Z, Kojić M, Medić D,* et al. Molecular Epidemiology of Colistin-Resistant, Carbapenemase-Producing Klebsiella pneumoniae in Serbia from 2013 to 2016. Antimicrob Agents Chemother 2017; 61(5): e02550–16. DOI: 10.1128/AAC.02550-16.
36. *Trudić A, Jelesić Z, Mihajlović-Ukropina M, Medić D, Zivlak B, Gusman V,* et al. Carbapenemase production in hospital isolates of multidrug-resistant Klebsiella pneumoniae and Escherichia coli in Serbia. Vojnosanit Pregl 2017; 74(8): 715–21. DOI**:** 10.2298/VSP150917260T.
37. *Šuto S, Bedenić B, Likić S, Kibel S, Anušić M, Tičić V,* et al. Diffusion of OXA-48 carbapenemase among urinary isolates of Klebsiella pneumoniae in non-hospitalized elderly patients. BMC Microbiol 2022; 22(1): 30. DOI: 10.1186/s12866-022-02443-y.
38. *Gajdács M, Ábrók M, Lázár A, Jánvári L, Tóth Á, Terhes G,* et al. Detection of VIM, NDM and OXA-48 producing carbapenem resistant Enterobacterales among clinical isolates in Southern Hungary. Acta Microbiol Immunol Hung 2020; 67(4): 209–15. DOI: 10.1556/030.2020.01181.
39. *Melinte V, Radu MA, Văcăroiu MC, Mîrzan L, Holban TS, Ileanu BV,* et al. Epidemiology of Carbapenem-Resistant Klebsiella Pneumoniae Co-Producing MBL and OXA-48-Like in a Romanian Tertiary Hospital: A Call to Action. Antibiotics (Basel) 2025; 14(8): 783. DOI: 10.3390/antibiotics14080783.
40. *Ludden C, Lötsch F, Alm E, Kumar N, Johansson K, Albiger B,* et al. Cross-border spread of blaNDM-1- and blaOXA-48-positive Klebsiella pneumoniae: a European collaborative analysis of whole genome sequencing and epidemiological data, 2014 to 2019. Euro Surveill 2020; 25(20): 2000627. DOI: 10.2807/1560-7917.ES.2020.25.20.2000627.
41. *Chatzidimitriou M, Kavvada A, Kavvadas D, Kyriazidi MA, Eleftheriadis K, Varlamis S,* et al. Carbapenem-resistant Klebsiella pneumoniae in the Balkans: Clonal distribution and associated resistance determinants. Acta Microbiol Immunol Hung 2024; 71(1): 10–24. DOI: 10.1556/030.2024.02230.
42. *Corbella L, Fernández-Ruiz M, Ruiz-Ruigómez M, Rodríguez-Goncer I, Silva JT, Hernández-Jiménez P,* et al. Prognostic factors of OXA-48 carbapenemase-producing Klebsiella pneumoniae infection in a tertiary-care Spanish hospital: A retrospective single-center cohort study. Int J Infect Dis 2022; 119: 59–68. DOI: 10.1016/j.ijid.2022.03.025.

Received on October 23, 2025
Revised on December 4, 2025
Revised on December 15, 2025
Accepted on December 24, 2025
Online First March 2026