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Article

Blood Stream Infections due to Carbapenem-Resistant Gram-Negative Bacilli, Producing *Klebsiella pneumoniae* Carbapenemase or New Delhi Metallo- β -Lactamase: A Single-Center, Retrospective, Comparative Study in a High-Prevalence Area

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Abstract: Blood stream infections (BSI) due to carbapenem-resistant gram-negative bacilli (CR-GNB), especially *Klebsiella pneumoniae* carbapenemase (KPC) and New-Delhi metallo- β -lactamase (NDM) producers, are a major concern of public health, as they are associated with high morbidity and mortality. We conducted a single-center, retrospective, observational study at a tertiary-care, teaching Greek hospital, to determine the incidence, 30-day and overall, in-hospital, crude mortality, risk factors and efficacy of best available treatment options in patients with BSIs from KPC- or NDM-producing CR-GNB. During the one-year study period, from 1st January to 31st December 2023, we recorded 133 episodes of BSIs in 118 unique patients; 79 episodes were due to KPC-producing and 54 to NDM-producing CR-GNB. Our study showed relatively high incidence of BSIs by both KPC- and NDM-producing CR-GNB (0.49 and 0.31 episodes per 1000 patient-days respectively). We found high 30-day crude mortality rates for patients with BSIs due to KPC- producing CR-GNB (26.4 %), and NDM-producing CR-GNB (37%), while BSIs due to NDM-producing *P. aeruginosa* had the highest 30-day (47.1%) and overall (70.6%) in-hospital, crude mortality. Beta-lactam/beta-lactamase inhibitors as monotherapy or in combination with other active agents used to treat KPC-producing CR-GNB were associated with improved survival rates ($p=0.009$).

Keywords: Blood stream infections; carbapenem-resistant gram-negative bacilli; *Klebsiella pneumoniae* carbapenemase; New-Delhi metallo- β -lactamase

1. Introduction

Carbapenem-resistant gram-negative bacilli (CR-GNB), especially those producing *Klebsiella pneumoniae* carbapenemases (KPCs) and New-Delhi metallo- β -lactamases (NDMs) represent a major public health problem [1]. KPCs are β -lactamases produced by gram-negative microorganisms that can hydrolyze nearly all β -lactams, including carbapenems, rendering them resistant to most available antibiotic options. First identified in the USA in 1996 [2], these enzymes have since spread in many countries around the world. NDMs are enzymes that belong to the greater family of metallo-

β -lactamases (MBLs). These enzymes require zinc to function and can break down almost all β -lactamic antibiotics including carbapenems [3].

Greece is an endemic area for nosocomial infections due to KPC-producing gram-negative bacteria, while the emergence of NDM-producing *K. pneumoniae* in Greek hospitals has been described in 2013 [4–6,8]. An increase in the incidence of infections due to KPC- and NDM-producers has been observed in the post-COVID-19 years in Greek hospitals [9,10].

Infections caused by CR-GNB have been proved to be a high priority problem of global health, since they are associated with high morbidity and mortality rates and remain a challenge for clinicians in daily practice [11]. Mortality rates of patients with blood stream infections (BSI) caused by CR-GNB, especially KPC-1 producers, have been reported to be as high as 53.2% [12]. Carbapenem-resistant *P. aeruginosa* mortality rates vary ranging from 6% to 32.8% [13,14]. The European Antimicrobial Resistance Surveillance Network (EARS-Net) in 2022 has reported a 49.7% increase in the incidence of carbapenem-resistant *K. pneumoniae* blood stream infections, compared to 2019 (3.26 cases per 100.000 population in 2022 vs. 2.18 cases per 100.000 population in 2019) [7].

Little is known about mortality rates caused by specific CR-GNB. Understanding the epidemiology, risk factors, outcomes, and the efficacy of existing treatment options is crucial for designing strategies to tackle the problem of CR-GNB in the clinical setting. The aim of this single-center retrospective, observational, real-life study was to analyze the epidemiology and outcomes, especially in-hospital mortality rates, of BSIs caused by NDM- and KPC-producing CR-GNB, as well as to identify possible risk factors and efficacy of treatment options, in a high-prevalence setting.

2. Results

We identified 133 episodes of bacteremia due to KPC- and NDM-producing CR-GNB in 118 unique patients, with a mean age of 67.8 ± 14.7 years. Among them 71 (60.2%) were male. Ten patients had more than one episode of bacteremia during their hospital stay. Seven patients had 2 episodes and two patients had 3 episodes of bacteremia. One patient had 5 distinct episodes of bacteremia from KPC-producing *K. pneumoniae*.

Of the 118 unique patients, 72 (61%) had BSI due to KPC-producing CR-GNB (all isolates belonged to the *Enterobacteriaceae* order) and 46 (39%) had NDM-producing CR-GNB isolated; among them 17/46 (37%) *Pseudomonas* isolates and 29/46 (63%) *Enterobacteriaceae* isolates. During the one-year study period, the occurrence rate of BSIs caused by NDM-producers was 0.31 per 1000 patient-days, whereas BSIs due to KPC-producing CR-GNB had a higher incidence rate of 0.49 per 1000 patient-days. The incidence of CR-GNB BSIs, including both KPC- and NDM-producers, reached a rate of 0.69 per 1000 patient-days.

Patient demographics and basic characteristics along with comparisons between the three types of isolates are shown on Table 1. Sixty-one out of the 118 study patients met the primary endpoint, yielding an overall crude in-hospital mortality of 51.7%. Overall, in-hospital mortality amongst patients with NDM-producing *Pseudomonas* was 70.6% compared to NDM-producing CR-GNB at 51.7% and KPC-producing CR-GNB at 47.2%, a difference not meeting statistical significance overall ($p=0.222$). Similarly, comparing in pairs NDM vs. KPC patients, overall, in-hospital mortality did not differ (51.7% vs 47.2% respectively, $p=0.826$), nor was there a difference between NDM-producing *Pseudomonas* and *Enterobacterales* patients (70.6% vs 51.7% respectively, $p=0.235$). The 30-day, after the index culture, crude in-hospital mortality was 40.7%, with BSIs from NDM-producing *P. aeruginosa* having the highest mortality (47.1%). We also calculated the mortality rates of all NDM-producing CR-GNB and KPC-producing CR-GNB and found similarly high rates (37% vs 26.4%, $p=0.305$).

Table 1. Basic characteristics of study participants and comparison between the three types of bacterial isolates.

	Total N=118	<i>Pseudomonas</i> NDM N=17	CREs NDM N=29	CREs KPC N=72	P
Male gender	71 (60.2)	9 (52.9)	20 (69)	42 (58.3)	0.495
Age (years)	67.8±14.7	73.1±13.8	73±15.7	64.5±13.7	0.009
Cancer history	53 (44.9)	3 (17.6)	9 (31)	41 (56.9)	0.003
LOS before index culture (days)	7 [1–19]	11 [1–20]	1 [0–6]	12 [2–25]	0.011
Ward					0.031
Medical	88 (74.6)	11 (64.7)	23 (79.3)	54 (75)	
Surgical	19 (16.1)	1 (5.9)	4 (13.8)	14 (19.4)	
ICU	11 (9.3)	5 (29.4)	2 (6.9)	4 (5.5)	
Hospital acquired infection	93 (78.8)	2 (11.8)	20 (69)	58 (80.6)	0.257
Central venous catheter	65 (55.1)	10 (71.4)	14 (70)	41 (71.9)	0.987
Foley catheter	67 (56.8)	13 (92.9)	15 (75)	39 (68.4)	0.175
Mechanical ventilation	14 (11.9)	5 (33.3)	4 (20)	5 (8.5)	0.042
Previously in ICU	20 (16.9)	6 (35.3)	4 (13.8)	10 (13.9)	0.093
Antibiotic resistance					
Colistin	37 (31.4)	1 (5.9)	15 (51.7)	21 (29.2)	0.004
Meropenem	116 (98.3)	17 (100)	28 (96.6)	71 (98.6)	0.648
Aztreonam	107 (90.7)	10 (58.8)	25 (86.2)	72 (100)	<0.001
Treatment					
Colimycin	57 (48.3)	15 (88.2)	19 (65.5)	23 (31.9)	<0.001
Ceftolozane/ tazobactam	3 (2.5)	1 (5.9)	1 (3.4)	1 (1.4)	0.536
Meropenem	20 (16.9)	7 (41.2)	7 (24.1)	6 (8.3)	0.003
Ceftazidime/ avibactam	49 (41.5)	3 (17.6)	1 (3.4)	45 (62.5)	<0.001
Meropenem/ vaborbactam	10 (8.5)	0 (0)	2 (6.9)	8 (11.1)	0.315
Imipenem/ relebactam	5 (4.2)	1 (5.9)	3 (10.3)	1 (1.4)	0.121
Tigecycline	15 (12.7)	1 (5.9)	7 (24.1)	7 (9.7)	0.095
Active Drugs					0.001
None	28 (23.7)	2 (11.8)	14 (48.3)	12 (16.7)	
One	71 (60.2)	15 (88.2)	10 (34.5)	46 (63.9)	
Two	19 (16.1)	0 (0)	5 (17.2)	14 (19.4)	
Laboratory values					
White blood cells (10 ³ /μl)	8.1 [3-16.2]	8.2 [4-16.4]	11.9 [5.2-22.1]	7.3 [1.4-13.4]	0.071
Neutrophils (10 ³ /μl)	6.7 [2.2-14.2]	6.6 [2.4-14.6]	11.2 [4.5-18]	5.6 [1-12.3]	0.067
Neutropenia	23 (19.5)	2 (11.8)	3 (10.3)	18 (25)	0.167
Gamma globulins (g/l)	9.8 [6.8-13]	10 [7.7-12]	10 [6.7-16]	9.7 [6.1-13]	0.713
Hypogamma-globulinemia	24 (20.3)	1 (11.1)	6 (31.6)	17 (31.5)	0.447
Albumin (g/l)	27.1 [22.9-32.3]	27.3 [23.8-33]	26.8 [23-33.9]	27.1 [22.2-32]	0.845
Recurrence of Bacteremia	10 (8.5)	2 (11.8)	2 (6.9)	6 (8.3)	0.847
LOS after culture (days)	13 [3–24]	14 [4–31]	12 [3–26]	14 [5–24]	0.976
Total LOS (days)	24 [12-43]	23 [15-49]	18 [9-37]	25 [13-46]	0.523
In-hospital mortality	61 (51.7)	12 (70.6)	15 (51.7)	34 (47.2)	0.222
30-day mortality	48 (40.7)	8 (47.1)	12 (41.4)	28 (38.9)	0.824

Data is presented as N (%) or mean±SD or median [IQR 25-75]. P-values in bold denote statistical significance. LOS: length of stay, ICU: Intensive Care Unit,.

We further attempted to identify risk factors predicting in-hospital mortality within the NDM and the KPC populations. Table 2 presents differences amongst survivors and non-survivors in the NDM group. In Cox-regression survival analysis, neither surgical ward admission (HR 0.72, 95%CI 0.21-2.52, p=0.608) nor ICU (HR 1.75, 95%CI 0.69-4.41, p=0.236) conferred greater risk of death

compared to medical wards. Similarly in Cox-regression, central venous catheter (HR 2.8, 95%CI 0.63-12.37, p=0.174), foley catheter (HR 3.76, 95%CI 0.49-28.86, p=0.204), mechanical ventilation (HR 2.19, 95%CI 0.91-5.28, p=0.081) and previous ICU admission (HR 1.28, 95%CI 0.55-2.97, p=0.573) did not increase in-hospital mortality risk.

Table 2. Comparison between survivors and non-survivors in the NDM population.

	Survivors N=19	Non-Survivors N=27	p
Male gender	12 (63.2)	17 (63)	1
Age (years)	74.5±15.2	72±14.9	0.579
Cancer history	7 (36.8)	5 (18.5)	0.19
LOS before index culture (days)	1 [0-8]	4 [1-15]	0.261
Ward			0.05
Medical	17 (89.5)	17 (63)	
Surgical	2 (10.5)	3 (11.1)	
ICU	0 (0)	7 (25.9)	
Hospital acquired infection	13 (68.4)	22 (81.5)	0.484
Central venous catheter	5 (38.5)	19 (90.5)	0.002
Foley catheter	7 (58.3)	21 (95.5)	0.014
Mechanical ventilation	0 (0)	9 (40.9)	0.013
Previously in ICU	1 (5.3)	9 (33.3)	0.031
Bacterial isolate			0.235
Pseudomonas	5 (26.3)	12 (44.4)	
Enterobacterales	14 (73.7)	15 (55.6)	
Antibiotic resistance			
Colistin	8 (42.1)	8 (29.6)	0.531
Meropenem	19 (100)	26 (96.3)	1
Aztreonam	11 (57.9)	24 (88.9)	0.032
Treatment			
Colimycin	14 (73.7)	20 (74.1)	1
Ceftolozane/ tazobactam	1 (5.3)	1 (3.7)	1
Meropenem	5 (26.3)	9 (33.3)	0.749
Ceftazidime/ avibactam	2 (10.5)	2 (7.4)	1
Meropenem/ vaborbactam	0 (0)	2 (7.4)	0.504
Imipenem/ relebactam	1 (5.3)	3 (11.1)	0.632
Tigecycline	3 (15.8)	5 (18.5)	1
Active Drugs			0.970
None	7 (36.8)	9 (33.3)	
One	10 (52.6)	15 (55.6)	
Two	2 (10.5)	3 (11.1)	
Laboratory values			
White blood cells (10 ³ /μl)	9.9 [5.1-18.1]	10.3 [4.2-20.5]	0.885
Neutrophils (10 ³ /μl)	8.6 [4.5-15.9]	8.6 [2.5-17.9]	0.964
Neutropenia	1 (5.3)	4 (14.8)	0.387
Gamma globulins (g/l)	11 [6.6-13.3]	9.6 [7.2-20]	0.872
Hypogammaglobulinemia	4 (28.6)	3 (21.4)	1
Albumin (g/l)	31.3 [24.3-36.6]	25.3 [22.4-29.5]	0.029
Recurrence of Bacteremia	1 (5.3)	3 (11.1)	0.632
LOS after culture (days)	16 [10-21]	9 [2-33]	0.32
Total LOS (days)	20 [10-33]	23 [12-63]	0.482

Data is presented as N (%) or mean±SD or median [IQR 25-75]. P-values in bold denote statistical significance. LOS: length of stay, ICU: Intensive Care Unit.

Differences amongst survivors and non-survivors in the KPC group are presented in Table 3. In cox-regression analysis, having a hospital-acquired infection did not increase in-hospital mortality risk (HR 1.89, 95%CI 0.57-6.31, $p=0.3$). Use of beta-lactam/beta-lactamase inhibitors (BLBLIs) in the KPC population, however, significantly reduced the risk of death (HR 0.21, 95%CI 0.1-0.43, $p<0.001$). Kaplan Meier survival function by BLBLI use ($p=0.009$) is displayed in Figure 1.

Table 3. Comparison between survivors and non-survivors in the KPC population.

	Survivors N=38	Non-Survivors N=34	p
Male gender	21 (55.3)	21 (61.8)	0.637
Age (years)	62.7±11.3	66.5±15.9	0.244
Cancer history	21 (55.3)	20 (58.8)	0.815
LOS before index culture (days)	9 [1-18]	13 [4-31]	0.057
Ward			0.186
Medical	27 (71.1)	27 (79.4)	
Surgical	10 (26.3)	4 (11.8)	
ICU	1 (2.6)	3 (8.8)	
Hospital acquired infection	27 (71.1)	31 (91.2)	0.039
Central venous catheter	16 (59.3)	25 (83.3)	0.075
Foley catheter	15 (55.6)	24 (80)	0.086
Mechanical ventilation	1 (3.7)	4 (12.5)	0.362
Previously in ICU	5 (13.2)	5 (14.7)	1
Antibiotic resistance			
Colistin	8 (21.1)	13 (38.2)	0.127
Meropenem	38 (100)	33 (97.1)	0.472
Treatment			
Colimycin	9 (23.7)	14 (41.2)	0.134
Ceftolozane/tazobactam	1 (2.6)	0 (0)	1
Meropenem	3 (7.9)	3 (8.8)	1
Ceftazidime/avibactam	30 (78.9)	15 (44.1)	0.003
Meropenem/vaborbactam	4 (10.5)	4 (11.8)	1
Imipenem/ relebactam	0 (0)	1 (2.9)	0.472
Tigecycline	2 (5.3)	5 (14.7)	0.243
Any BLBLI	33 (86.8)	20 (58.8)	0.009
Active Drugs			0.083
None	3 (7.9)	9 (26.5)	
One	28 (73.7)	18 (52.9)	
Two	7 (18.4)	7 (20.6)	
BLBLI monotherapy	27 (81.8)	13 (65)	0.2
Laboratory values			
White blood cells ($10^3/\mu\text{l}$)	8.2 [4.6-12.2]	4.8 [0.8-15.4]	0.391
Neutrophils ($10^3/\mu\text{l}$)	6.7 [3.4-10.9]	3.9 [0.5-14.2]	0.369
Neutropenia	7 (18.4)	11 (32.4)	0.188
Gamma globulins (g/l)	10.5 [8.1-17]	8.3 [5.9-11]	0.098
Hypogammaglobulinemia	5 (19.2)	12 (42.9)	0.082
Albumin (g/l)	30 [24.1-33.7]	24.1 [20.4-30.8]	0.011
Recurrence of Bacteremia	3 (7.9)	3 (8.8)	1
LOS after culture (days)	17 [10-25]	9 [1-24]	0.045
Length of stay (days)	23 [17-33]	28 [9-55]	0.743

Data is presented as N (%) or mean±SD or median [IQR 25-75]. P-values in bold denote statistical significance. LOS: length of stay, ICU: Intensive Care Unit, BLBLI: beta-lactam/beta-lactamase inhibitor.

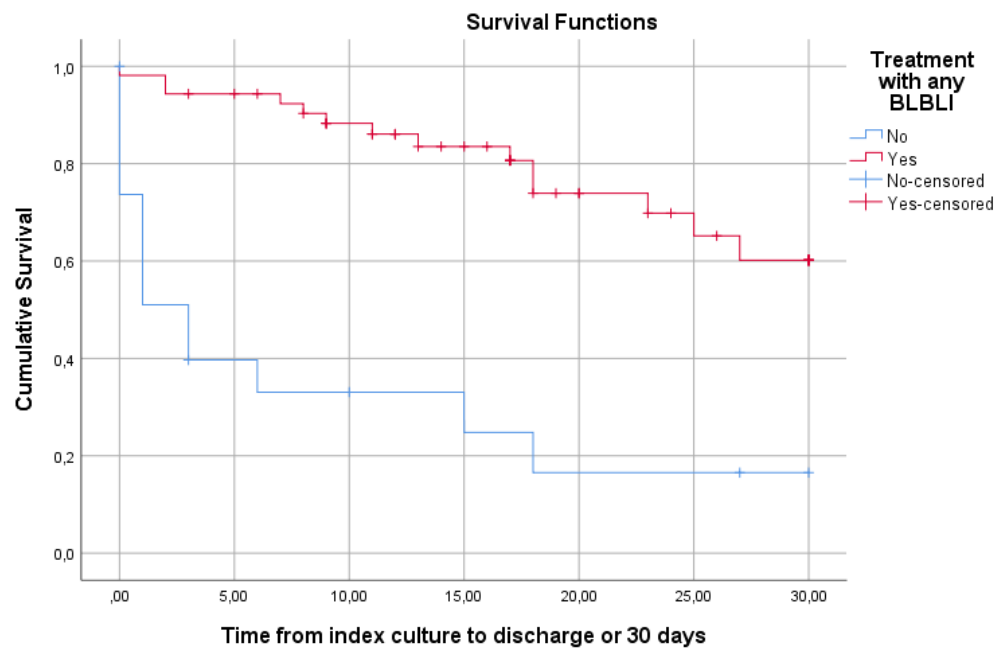


Figure 1. Kaplan Meier survival analysis in the *Klebsiella pneumoniae* carbapenemase population by use of beta-lactam/beta-lactamase inhibitor, $p=0.009$.

With regards to the 133 microbial isolates, 27 (17.3%) were *P. aeruginosa* and 110 (82.7%) *Enterobacteriales*, mainly *K. pneumoniae* (106/110). Table 4 presents a comparison between the NDM-producing and KPC-producing isolates. Forty-two of the 54 NDM-producing isolates (77.8%) were resistant to aztreonam; only one out of 17 (5.9%) *Pseudomonas* isolates was resistant to colistin.

Table 4.

	NDM N=54(%)	KPC N=79(%)	p
Microbes			<0.001
<i>P. aeruginosa</i>	23 (42.6)	0 (0)	
<i>K. pneumoniae</i>	30 (55.6)	76 (95.2)	
<i>E. coli</i>	1 (1.9)	2 (2.5)	
<i>K. aerogenes</i>	0 (0)	1 (1.3)	
Antibiotic resistance			
Colistin	17 (31.5)	22 (27.8)	0.7
Meropenem	53 (98.1)	78 (98.7)	1
Aztreonam	42 (77.8)	79 (100)	<0.001

P-values in bold denote statistical significance.

3. Discussion

This retrospective study was designed and conducted to evaluate the incidence, in-hospital mortality, efficacy of therapeutic options and risk factors of BSIs caused by NDM- and KPC producing CR-GNB. We identified 79 episodes of BSIs due to KPC-producing and 54 episodes of BSIs due to NDM-producing CR-GNB during one year. We found that the incidence of BSIs linked to KPC-producers was higher compared to the incidence of BSIs caused by NDM-producing (*P. aeruginosa* and *Enterobacteriaceae* included) CR-GNB. Overall and 30-day, crude in-hospital mortality was observed to be higher amongst patients with BSIs due to NDM-producing *P. aeruginosa* compared to

those with BSIs due to KPC- or NDM-producing *Enterobacteriaceae*, although the difference did not reach statistical significance. The use of BLBLI as monotherapy or in combination with other active agents was associated with better survival rates for patients with KPC-producing CR-GNB.

Our study showed relatively high incidence of BSIs caused by both KPC and NDM producers. The incidence of KPC-producers was comparatively higher (0.49 per 1000 patient-days), indicating an endemic status in our country, aligning with our existing knowledge [7,17]. Other studies conducted in Greek hospitals or hospitals in countries endemic for CREs have reported the incidence of BSIs caused by carbapenem-resistant *K. pneumoniae* to range from 0.092 to 0.59 per 1000 patient-days [18–21]. Here we report a similar, albeit lower, incidence of BSIs caused by NDM-producing CR-GNB, a finding suggesting that those difficult to treat pathogens became endemic as well. Our study is, to our knowledge, the first to document similar incidence of BSIs caused by NDM- and KPC-producing CR-GNB.

An important finding of our study is the high in-hospital mortality observed among study patients. BSIs due to both NDM- and KPC-producing CR-GNB exhibited high 30-day and overall, in-hospital crude mortality rates. Although not statistically significant, BSIs due to NDM-producing *P. aeruginosa* were associated with the highest mortality (overall crude in-hospital mortality 70.6% and 30-day in-hospital mortality 47.1%). Similarly, unacceptably high mortality rates have been reported by other researchers from endemic areas. In the ALARICO study, a prospective multicenter study conducted in Italy, a region endemic for CR-GNB, the 30-day mortality attributable to metallo- β -lactamase producing (MBL) *Enterobacterales*, (most of which were NDM-producers), and KPC-producing *Enterobacterales*, was 36.4% and 26.6% respectively [13]. In another prospective, observational 3-year study from Italy, 30-day mortality for MBL *Enterobacterales* was reported to be 29.7%, including NDM- and VIM-producers [22], emphasizing the need for early antimicrobial treatment with active agents to improve the outcomes. Our study reported similar, albeit higher, 30-day in-hospital mortality rates, possibly attributed to the limited availability of active antibiotics, the hospital's high proportion of immunocompromised patients and inadequate implementation of infection control measures. Remarkably, 19 patients did not receive any active antibiotics, yet some survived possibly due to timely and efficient source control and better overall patient health status. Limited data are available for the mortality rates of BSIs caused by NDM-producing *P. aeruginosa*. Nevertheless, it is well established that carbapenem-resistant *P. aeruginosa* is a global threat with concerning levels of morbidity and mortality [13,14]. Our study is the first to report an overall crude mortality rate of BSIs due to NDM-producing *P. aeruginosa* as high as 70.6%.

In our risk factor analysis, we found that patients with BSIs caused by NDM-producers tended to be significantly older, whereas patients with BSIs due to KPC-producing CR-GNB were more likely to have an underlying malignancy. The length of stay before index culture was significantly lower in the NDM group (1 [0–6], $p=0.011$). This result, combined with older age, indicates that this group of patients tend to have more frequent previous hospitalizations or to be more often residents in long-term care facilities. In the NDM group, the Cox-regression survival analysis showed that risk factors for adverse outcomes were the presence of central venous catheter and/or urinary catheter, mechanical ventilation, and prior admission in the intensive care unit, albeit these factors, did not achieve statistical significance. Within the same group of patients, the use of one or two concomitant active agents did not result in better survival rates. Researchers have shown that ceftazidime-avibactam (CZA-AVI) in combination with aztreonam is an effective treatment for patients with MBL infections, leading to a reduction in mortality rates; hence this combination has been included in current guidelines as first line option [23–27]. Falcone and colleagues in Italy studied MBL infections in a prospective study spanning 3 years [22]. In this study, 345 patients with MBL infections were included, most of whom had BSI (199/345). The authors concluded that the combination of ceftazidime-avibactam plus aztreonam lowers mortality rates. In our study, we did not observe better outcomes for patients with BSIs due to NDM-producing CR-GNB, treated with one or two active antimicrobials, possibly due to the small number of cases included, the lack of aztreonam in our country, which restricted its administration in most cases, and the high in vitro resistance rates [42/54 (77.8%)] of NDM-producing strains to aztreonam.

Within the KPC group, most patients were treated with a BLBLI. Noteworthy is the fact that all KPC strains were found to be resistant to aztreonam. Compared to non-survivors, in the KPC group the use of BLBLIs has resulted in better survival rates (33/38 (86.8%) vs 20/34 (58.8%), $p=0.009$), which is consistent with current knowledge. Several prospective and retrospective studies have shown the efficacy of ceftazidime-avibactam as first-line therapy against KPC Enterobacterales [27–30]. The use of ceftazidime-avibactam has been documented to improve survival rates for most infections due to KPC-producing CR-GNB, apart from lower respiratory tract infections. Newer agents, such as meropenem/varbobaactam and imipenem/relebactam have been used for CZA-AVI resistant strains [31,32], yielding favourable outcomes.

4. Materials and Methods

4.1. Study Design

This is a single-center, retrospective study conducted at the General Hospital of Athens Laiko, a 580-bed, university-affiliated, teaching hospital, serving the greater area of Athens. All consecutive adult patients, with confirmed BSI due to KPC- or NDM-producing CR-GNB, during a one-year period, from 1st January 2023 to 31st December 2023, were included in the study. Patients with polymicrobial BSIs caused by multiple GNB, or by a CR-GNB plus gram-positive bacteria or fungi were excluded. We retrospectively reviewed the electronic and/or chart files of the patients, and by using a preformed clinical report form we collected data including demographics, medical history and underlying diseases, laboratory parameters at the time when index cultures were drawn, length of stay in the hospital, microbiological data and outcomes. All retrieved data were entered in an electronic database for statistical analysis. The primary outcome was the 30-day and overall, in-hospital crude mortality rate for patients with BSIs caused by different categories of CR-GNB and the comparison of BSIs due to KPC-producing CR-GNB to BSIs due to NDM-producing CR-GNB. Secondary outcomes included length of stay in the hospital and BSI recurrence

4.2. Definitions

BSI was defined according to the Centers for Disease Control and Prevention/National Healthcare Safety Network criteria [16]. BSIs were considered as hospital-acquired when positive index cultures were drawn at least 48 hours after patient's admission to the hospital or if patients had been hospitalized for any reason within the previous 7 days, in accordance with the ECDC definitions [7]. Antibiotic susceptibilities were measured by MicroScan autoSCAN-4 Microbiology System (Beckman Coulter; Brea, California, USA). KPC and/or NDM detection was performed using NG-Test Carba 5 (NG Biotech; Guipry, France), a rapid in vitro multiplex immunoassay. Medications used were considered active, if the isolated microorganism was susceptible to them. Patients were considered neutropenic when their neutrophil counts were $< 1000 /\text{mL}$ and were described as hypogammaglobulinemic when their measured gamma globulin levels were below 7g/L. For patients with multiple episodes of bacteremia during the study period, we used the first recorded episode as the index case.

4.3. Statistical Analysis

Categorical variables are expressed by absolute values and relative frequencies. Continuous variables are measured by mean and standard deviation (SD), for normally distributed variables, or median and interquartile range (IQR), for non-normally distributed variables. Normality was tested with the Shapiro-Wilks test and equality of variances by Levene's test. Group comparisons were performed using the Mann-Whitney U test for continuous non-normally distributed variables and Student's t-test for normally distributed variables. Group comparisons for categorical variables were performed using the Chi-square and ANOVA tests. Survival analysis was carried out using Kaplan-Meier curves and univariable Cox regression. The level of statistical significance was set to <0.05 . All statistical analysis was performed using the IBM SPSS Statistics for Windows, version 25.0 (2017, IBM Corp., Armonk, NY, USA).

Our study has limitations, with the most important being its retrospective nature. Additionally, the results should be read with caution given the small number of cases included in the study. Nevertheless, this is, to our knowledge, the first study to describe comparatively the occurrence and mortality rates of BSIs caused by NDM- and KPC-producers in our country.

5. Conclusions

In conclusion, in the present retrospective observational study spanning one year, we found high incidence of BSIs due to NDM- and KPC-producing CR-GNB. We observed a high mortality for both KPC- and NDM-producing CR-GNB with the highest in-hospital mortality observed amongst patients with NDM-producing *P. aeruginosa*. Given the limited availability of effective antibiotic agents and the rising resistance to existing treatment options in our country for NDM strains, the finding that using one or two active antibiotics for NDM producers does not yield significant differences is noteworthy. Finally, in this study we confirmed current knowledge about BLBLIs as the best available treatment option for BSIs due to KPC-producing CR-GNB.

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Informed Consent Statement: Patient consent was waived due to the non-interventional nature of the study.

Data Availability Statement: Raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher, upon request.

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

Abbreviations

CR-GNB: carbapenem-resistant gram-negative bacilli, BSI: blood stream infections, KPC: *Klebsiella pneumoniae* carbapenemase, NDM: New-Delhi metallo- β -lactamase, MBL: metallo- β -lactamases, BLBLI: beta-lactam/beta-lactamase inhibitors, CZA/AVI: ceftazidime/avibactam

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