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Incidence of hospital-acquired infections due to carbapenem-resistant *Enterobacterales* and *Pseudomonas aeruginosa* in critically ill patients in Italy: a multicentre prospective cohort study

Gennaro De Pascale[1,2†], Andrea Cortegiani[3,4*†], Matteo Rinaldi[5,6], Massimo Antonelli[1,2], Sergio Cattaneo[7], Maurizio Cecconi[8,9], Raffaele Cuffaro[10], Lidia Dalfino[11], Filomena Di Biase[12], Abele Donati[13,14], Francesca Romana Fasano[15], Teresa Fasciana[16], Giuseppe Foti[17,18], Antonella Frattari[19], Roberto Fumagalli[20,21], Massimo Girardis[22], Leonardo Gottin[23], Alessia Mattei[24], Marta Milazzo[3], Giorgia Montrucchio[25,26], Daniela Pasero[27,28], Fabio Picciafuochi[29], Emanuela Sensi[30], Giuseppe Servillo[31], Maria Alejandra Vidal Pereira[15], Teresa Spanu[32†], Pierluigi Viale[5,6†] and on behalf of the INCREASE-IT Study Group

Abstract

Background Carbapenem-Resistant Gram-Negative Bacteria, including Carbapenem-Resistant *Enterobacterales* (CRE) and Carbapenem-Resistant *Pseudomonas aeruginosa* (CRPA), are common causes of infections in intensive care units (ICUs) in Italy.

Objective This prospective observational study evaluated the epidemiology, management, microbiological characterization, and outcomes of hospital-acquired CRE or CRPA infections treated in selected ICUs in Italy.

Methods The study included patients with hospital-acquired infections due to CRE and CRPA treated in 20 ICUs from June 2021 to February 2023. The primary endpoint was the 1-year incidence of CRE/CRPA infections. Secondary endpoints included the rate of CRE/CRPA infections, mortality in ICU, infection outcome, and microbiological characterization.

Results Among 13,088 patients admitted over the 12-month study period across each of the 20 ICUs, 283 had CRE infections, and 138 had CRPA infections. The incidence of CRE and CRPA infections was 3.57 per 1000 patient days and 1.74 per 1000 patient days, respectively. The proportion of CRE and CRPA infections over the total number of infections due to *Enterobacterales* and *Pseudomonas aeruginosa* was 19.2% and 26.8%, respectively. Among 158

†Gennaro De Pascale and Andrea Cortegiani contributed equally and share first authorship.

†Teresa Spanu and Pierluigi Viale contributed equally to this work and share last authorship.

*Correspondence:
Andrea Cortegiani
andrea.cortegiani@unipa.it
Full list of author information is available at the end of the article

patients included in the full analysis, 98 (62%) had CRE infections and 60 (38%) had CRPA infections. Ventilator-associated pneumonia and bloodstream infections were the most common infections, occurring in 53.8 and 34.2% of cases. Empirical therapy targeting gram-negative pathogens resulted inappropriate in 59.2% of analysed patients (77/130). The overall crude mortality in ICU rate was 30.4%, with a higher rate in CRE patients (36.7%) than in CRPA patients (20.0%). Clinical success, including microbiological eradication, was achieved in 50.6% of cases. *Klebsiella pneumoniae* was observed as the predominant CRE species, and all CRE isolates, including metallo-β-lactamases-producing CRE (MBL-CRE), were susceptible to Aztreonam-Avibactam.

Conclusions These results highlight the high prevalence of CRE/CRPA infections in Italian ICUs and emphasize the need for enhanced prevention and surveillance strategies.

Keywords Carbapenem-Resistant *Enterobacterales*, *Pseudomonas aeruginosa*, Intensive care units, Italy

Introduction

Carbapenem Resistant-Gram Negative Bacteria (CR-GNB) are highly transmissible and have a high potential to cause outbreaks in healthcare settings [1], particularly in intensive care units (ICUs) [2]. As forecasted by the GBD 2021 Antimicrobial Resistance Study Group, an estimated 8.22 million deaths associated with antimicrobial resistance (AMR) could globally occur in 2050, imposing the strong need for interventions and novel antibiotic development to mitigate such a concerning scenario [2]. Nowadays, Carbapenem-Resistant *Enterobacterales* (CRE) and Carbapenem-Resistant *Pseudomonas aeruginosa* (CRPA) have been increasingly reported worldwide [3, 4]. In Europe, Italy shows an estimated burden of CRE and CRPA higher compared to other EU countries, with about one-third of the deaths associated with infections due to antibiotic-resistant bacteria in the EU occurring in Italy [5, 6], although with important variability across regions, hospitals, and even within different wards [7]. Data from the Italian National Surveillance System (2022) show that the incidence of CRE-related bloodstream infections (BSIs) rose compared to 2021 and the previous five years, with regional differences in prevalence and incidence [5].

CR-GNB infection is associated with high mortality rates and severe clinical outcomes, likely due to the limited availability of effective treatment options. In Italy, a recent survey [8] conducted in 15 Hospitals in the Northern regions has shown that carbapenemase-producing *K. pneumoniae* poses a major challenge for Italy's healthcare system and is associated with high rates of mortality and hospitalizations. In another study including patients with GNB BSIs from 19 Italian hospitals, carbapenem resistance was associated with an excess of mortality, with metallo-β-lactamase (MBL)-CRE carrying the highest risk of death, followed by CRPA [9].

Treating Multidrug-Resistant (MDR) GNB infections in critically ill patients is challenging. Resistance to many antimicrobial classes almost invariably reduces the probability of adequate empirical coverage, with possible unfavourable outcomes [9].

Based on findings from the latest epidemiological studies, this study aimed to generate up-to-date data regarding the epidemiology, management, microbiological characterization, and outcomes in patients with documented CRE and CRPA infections in selected ICUs in Italy. These data are crucial for guiding therapeutic strategies and preventing the further spread of these highly resistant pathogens in healthcare settings.

Patients and methods

Study design

This prospective, multicenter, non-interventional cohort study aimed to estimate the annual incidence of CRE and CRPA infections in 20 Italian ICUs. Adult patients diagnosed with hospital-acquired CRE or CRPA infections and receiving treatment in these ICUs were eligible. Hospital-acquired infection was defined as an infection occurring in any body site after ≥ 48 h following hospital admission, including those acquired both during ICU stay and prior to ICU admission. To be included in the study, the patient had to meet the criteria for any of the following microbiologically documented infections: bloodstream infections (BSI), urinary tract infections (UTI), hospital-acquired or ventilator-associated pneumonia (HAP/VAP), intra-abdominal complicated or uncomplicated infection (IAI), or other infections (e.g., meningitis, endocarditis, or skin/skin structure infections). Infection definitions are reported in the Supplementary Materials. Pregnant or lactating women and

patients included in any interventional study at the time of enrollment were not eligible.

Therapeutic strategies adopted by clinicians were based on routine clinical practice or standard practice guidelines for each ICU.

Participating investigators were asked to include all consecutive patients with CRE or CRPA infections attending the ICU, who were followed until one of the following end-of-study criteria occurred: death, discharge from ICU, infection resolution, a 30-day ICU stay, or consent withdrawal, whichever occurred first. The study period spanned one year from the initiation date at each site.

Primary endpoints

The primary endpoint was to determine the one-year incidence of CRE/CRPA infections in Italian ICUs, calculated as the total number of CRE/CRPA infections divided by the total number of patient days in each ICU over one year. Moreover, the incidence risk per ICU admission was calculated as the total number of CRE/CRPA infections divided by the total number of ICU admissions recorded during the one-year period from the start of the study.

Secondary endpoints

Secondary endpoints included: (i) the proportions of CR-GNB, calculated as the total number of patients with documented CRE/CRPA infections divided by the aggregate number of infections due to *Enterobacterales*/*Pseudomonas* in each ICU over the one-year observation period; (ii) clinical outcomes: morbidity indices and mortality in ICU (defined as mortality during ICU stay within the timeframe of the study); (iii) treatment patterns (including the frequencies and percentages of each antibiotic classes administered as monotherapy and combination therapy before study enrolment and after receiving antibiogram results); (iv) infection outcome defined by the rate of success of either cure (clinical improvement) and microbiological eradication (with a negative follow-up culture) or suspected eradication (no follow-up culture); (v) rate of failure, defined as death, clinical or microbiological failure, need for antibiotic treatment correction; (vi) microbiological characterization.

The appropriate empiric antimicrobial therapy was defined as the administration of at least one drug with in vitro and clinical activity against the isolated pathogens and initiated within the first 24 h.

Microbiology

After data collection and completion of the study, a microbiological analysis was performed on re-cultured samples in a central lab. Microbiological analyses were performed on 94 carbapenem-resistant *Enterobacterales* (CRE) and 52 carbapenem-resistant *Pseudomonas aeruginosa* (CRPA). All isolates were characterized by phenotypic antimicrobial susceptibility testing and whole-genome sequencing to identify resistance determinants. The minimum inhibitory concentrations (MICs) were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) version 14.0 breakpoints (https://eucast.org). For this study, *Enterobacterales* were considered CRE if resistant to meropenem or imipenem according to EUCAST breakpoints or if carbapenemase-producing (regardless of the MIC to carbapenems). *P. aeruginosa* resistant to meropenem and/or imipenem at the EUCAST breakpoints were phenotypically considered CRPA.

MICs of amikacin, amoxicillin, amoxicillin-clavulanic acid, aztreonam, cefepime, cefotaxime, ceftazidime, colistin, ertapenem, imipenem, meropenem, piperacillin-tazobactam, gentamicin, ciprofloxacin, and trimetoprim-sulphametoxazole were determined by broth microdilution method (BMD) using MDRO e PSE plates (Bruker Daltonics GmbH& Co. KG). We also obtained MICs for imipenem-relabactam and meropenem vaborbactam (both from E-Etest, bioMérieux, Marcy l'Etoile, France) and aztreonam avibactam (MIC TEST strips from Liofilchem, Teramo, Italy). Susceptibility to cefiderocol was evaluated using the disk diffusion method (Liofilchem) according to the EUCAST guidelines. *E. coli* ATCC 25922, *E. coli* ATCC 35218, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 25783 were included as quality control strains in all sessions. EUCAST (version 14.0, 2024) clinical breakpoints for *Enterobacterales* or *P. aeruginosa* were used to interpret MICs. Identification of carbapenemase genes was performed by sequencing all isolates with Illumina technology (Illumina, San Diego, CA, USA).

Statistical analysis

Statistical analyses and data processing were performed with SAS® software version 9.4 (SAS® Institute Inc., Cary, North Carolina, US) on a Windows 7 operating system. For continuous data, summary statistics were generated, including the number of observations, mean, standard deviation (SD), median, and range (minimum and maximum). Frequency distributions and percentages were presented for categorical data. All descriptive summaries were reported in the total sample and by infection type (CRE or CRPA).

Ethics

The study protocol was approved by the Ethics Committee of the coordinating centre (Fondazione Policlinico

Gemelli Ethic Committee, registry number 0002278/21) on 21st January 2021. The other participating centers followed the local ethical committees' requirements. Written informed consent was obtained from the patients (or their legally acceptable representative).

Results

Study cohort

The total study period spanned from June 2021 to February 2023. Among 13,088 patients admitted over the 12-month study duration across each of the 20 ICUs (79,246 patient-days), 283 had CRE infections, and 138 had CRPA infections, respectively, and were considered for the primary endpoint. A total of 158 patients met the inclusion criteria, with 98 having CRE infections (21 were colonized by a KPC-producing *Klebsiella pneumoniae*), and 60 with CRPA (Fig. 1). Of the 158 patients, 80 (50.6%) had an infection at admission, while 78 (49.4%) had an ICU-acquired infection.

Epidemiology

The incidence of CRE infections was 3.57 per 1000 patient days [95% CI: 3–4], while the incidence of CRPA infections was 1.74 per 1000 patient days [95% CI: 1–2]. The incidence risk per ICU admission of CRE infections was 2.2% (range 0.3–9.8%), and the incidence risk per ICU admission of CRPA infections was 1.1% (range 0.2–6.2%). Among all the infections, CRE accounted for 19.2% of *Enterobacterales* infections, and CRPA accounted for 26.8% of *P. aeruginosa* infections ($p < 0.001$ between subgroups).

Demographic and clinical characteristics of the study cohort

Table 1 summarizes the demographic and clinical characteristics of the included patients: 71.5% were males, with similar gender distribution in patients with CRE and CRPA. The median age was 61.5 years (interquartile range (IQR) [18–92]) and was slightly higher in patients with CRE (62.5 years, IQR [18–92]) than in those with CRPA (60 years, IQR [24–80]). Comorbidities such as obesity and diabetes were more common in patients with CRE.

VAP was the most common hospital-acquired infection, reported in 41.1% of cases, followed by BSIs, which accounted for 34.2% of infections. Among the BSIs, 29.6% were catheter related. Among the overall study population, 27.2% of patients presented with septic shock at enrolment.

The most common underlying conditions and predisposing factors included the presence of a urinary catheter at ICU admission (89.2% of patients), intubation or mechanical ventilation (81.6%), presence of a central venous catheter at ICU admission (58.2%), and sepsis at study enrolment (51.9%). The median Charlson comorbidity index in the overall population was 4.0 (IQR 0–24) and was higher in patients with CRE (median 4) compared to those with CRPA (median 3).

Figure 2 presents the classes of antibiotics against gram-negative bacteria administered as both empirical and targeted therapy in the overall population. A carbapenem-based regimen was the most frequent choice (75 patients, 47.5%), mainly associated with oxazolidinones (22 patients, 13.9%). Empirical therapy was evaluated in 144 patients; of them, 35 were managed with monotherapy, and 109 were treated with a wide-spectrum combination regimen. Considering only empirical treatment targeting gram-negative pathogens (130 patients), monotherapy was adopted in 87 (66.9%) patients, whereas a combination regimen was reported in 43 (33.1%) cases. Among them, rates of inappropriate empirical therapy were 66.7% and 44.2% for mono- and combo-regimens, respectively. Overall, empiric therapy resulted inappropriate in 59.2% of analysed patients (77/130). Notably, higher rates of inappropriateness were observed for CRE (74%), in particular, 73.1% for KPC-producing CRE and 80% for MBL-CRE, than CRPA (37.7%).

Infectious disease specialist consultations were provided daily in 15.2% of the 20 ICUs and on-demand consultations were offered in the remaining 84.8%.

Microbiological characterization

Table 2 shows the microbiological characterization of 146 isolates (94 CRE and 52 CRPA). Among the 94 CRE isolates, the most common species was *Klebsiella pneumoniae* (n = 87, 92.5%). Genomic analysis of these isolates revealed a predominance of ST 512 *K. pneumoniae* isolates (n = 32, 36.8%), followed by ST 307 isolates (n = 23, 36.8%) and ST 101 isolates (n = 21, 24.1%). The remaining isolates belonged to ST147 (n = 5, 3.5%), ST17 (n = 2, 2.3%), ST11 (n = 2, 2.3%), ST 1876 and ST 661 (one isolate each). Other *Enterobacterales* included *Enterobacter cloacae* complex (n = 3, 3.2%), *Klebsiella aerogenes* (n = 2, 2.1%), *Escherichia coli* (n = 1, 1.1%), and *Providencia stuartii* (n = 1, 1.1%). A total of 77 isolates (81.9%) carried bla_{KPC} genes, including genes encoding KPC-3 (59 isolates), KPC-2 (6 isolates), KPC-166 (6 isolates), KPC-167 (5 isolates), and KPC-184 (1 isolate). Thirteen CRE isolates carried MBL genes, including 8 bla_{NDM-1}, 3 bla_{VIM-1}, 1 bla_{VIM-1} plus bla_{KPC-2}, and 1 bla_{VIM-1} plus bla_{KPC-3}. Two isolates harbored $bla_{OXA-181}$ and 2 carried $bla_{OXA-181}$ plus bla_{KPC-3} (Table 2).

All isolated CRE were susceptible to aztreonam-avibactam (AZA). In addition, 91% of CRE isolates were susceptible to cefiderocol (FDC), 84% to

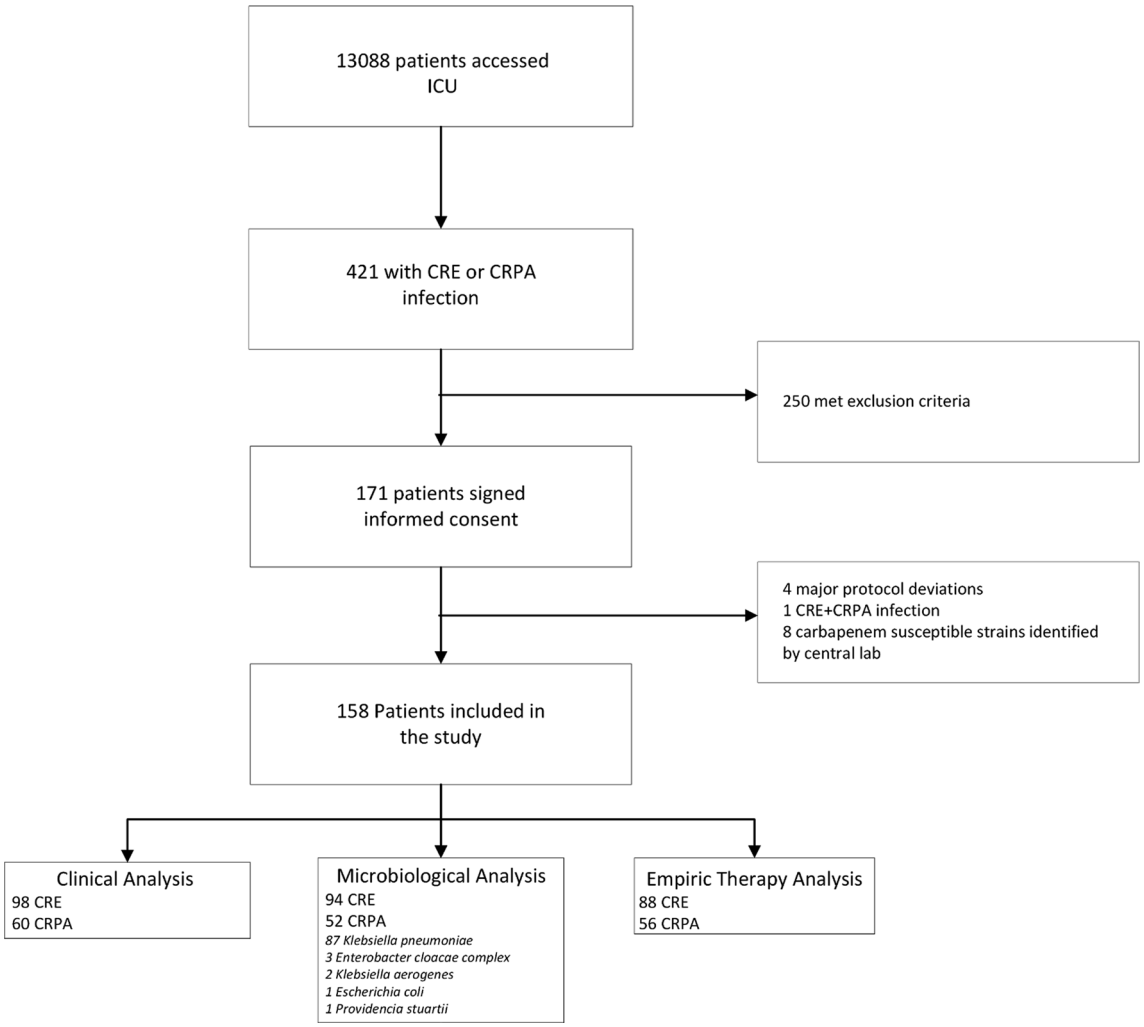

Fig. 1 Study flow-chart. Abbreviations: CRE, Carbapenem resistant *Enterobacterales;* CRPA, Carbapenem Resistant *Pseudomonas aeruginosa:* ICU, Intensive Care Unit

meropenem-vaborbactam (MVB), 83% to imipenem-relebactam (IMI-REL), 74% to ceftazidime/avibactam (CZA) and 74% to colistin (COL) (Table 3).

The activities of FDC, CZA, MVB, and IMI-REL varied according to the type of carbapenemase produced by the organism (Tables S1 and S2). Among 77 KPC-producing *Enterobacterales*, 100% were susceptible to aztreonam-avibactam (AZA), IMI-REL and MVB, 94% to FDC and 86% to CZA (Table S1). Eleven isolates, collected from in-patients at two ICUs in northern Italy, were resistant to CZA (MIC ≥ 64 mg/L) and susceptible to both imipenem and meropenem (MIC ≤ 0.5 mg/L). Six of them harbored the $bla_{\text{KPC-166}}$ gene, and five harbored the $bla_{\text{KPC-167}}$, two bla_{KPC} variants recently described in Italy. Strains harbouring $bla_{\text{KPC-167}}$ were also resistant to FDC. The 13 MBL-CRE were 100% susceptible to AZA, 82% susceptible to COL and 73% susceptible to FDC (Table S2).

Among the 52 CRPA isolates, 5 strains produced VIM-2 and 1 produced VIM-1 (Table 2). CRPA isolates showed similar susceptibility rates to CZA (85%) and C/T along with high susceptibility rates to cefiderocol (100%), colistin (100%), and imipenem-relebactam (88.5%) (Table 4).

Clinical outcomes

The overall mortality in ICU rate was 30.4%, significantly higher in patients with CRE (36.7%) than in patients with CRPA (20.0%) ($p = 0.026$). In patients with infections caused by KPC-producing CRE (N = 74), the mortality rate in the ICU was 37.8%, and the treatment failure rate was 54.1%. Similarly, in patients with infections due to New Delhi MBL (NDM)-producing CRE (N = 8), both

Table 1 Demographic and clinical characteristics of the study cohort

	Total cohort (n = 158)		**CRE (n = 98)**		**CRPA (n = 60)**		
Variables	No. of patients	% [IQR]	No. of patients	% [IQR]	No. of patients	% [IQR]	*P* value
Demographics and comorbidities							
Age (Median), years	61.5	[18–92]	62.5	[18–92]	60	[24–80]	0.070
Gender (male)	113	71.5	70	71.4	43	71	0.974
Ethnicity (Hispanic/Latino)	42	26.6	26	26.5	16	26.7	0.985
Recent hospitalization*	53	33.5	31	31.6	22	36.7	0.515
Recent stay in a LTCF*	13	8.2	7	7.1	6	10	0.526
Previous contact with CRE*	13	8.2	11	11.2	2	3.3	0.133
Previous contact with CRPA*	4	2.5	2	2	2	3.3	0.635
Previous colonization with CRE*	24	15.2	20	20.4	4	6.7	0.022
Previous infection with CRE*	6	3.8	3	3.1	3	5	0.674
Previous colonization with CRPA*	10	6.3	3	3.1	7	11.7	0.043
Previous infection with CRPA*	6	3.8	1	1	5	8.3	0.030
BMI ≥ 30 kg/m^2	33	22	26	25.5	7	11.7	0.026
Diabetes	42	26.6	31	31.6	11	18.3	0.066
Charlson index (Median)	4	[0–24]	4	[0–24]	3	[0–14]	0.070
Chronic kidney disease							
Renal impairment (overall)	56	35.44	43	43.9	13	21.6	0.005
Renal impairment Stage I	12	21.8	8	18.6	4	33.3	1.0
Renal impairment Stage II	5	9.1	5	11.6	0	0	0.157
Renal impairment Stage III	26	47.3	21	48.8	5	41.7	0.045
Renal impairment end-Stage	13	23.6	9	20.9	4	33.3	0.768
Immunosuppression**	81	51.2	42	42.8	39	65	0.007
Chronic liver disease							
Hepatic impairment	28	17.7	19	19.4	9	15.0	0.483
Clinical ICU presenting features							

Table 1 (continued)

	Total cohort (n = 158)		CRE (n = 98)			CRPA (n = 60)		
APACHE II score (Median)	19	[0–73]	19	[5–71]		16.5	[0–73]	0.375
SOFA score at ICU admission (Median)	7	[0–20]	7	[1–20]		7	[0–16]	0.625
SOFA score at Infection Onset (Median)	7	[0–19]	8	[0–19]		7	[0–13]	0.099
Pre-ICU Hospital LOS, days (Median)	4	[1–146]	6	[1–133]		3	[1–146]	0.959
ICU admission, medical	102	64.6	64	65.3		38	63.3	0.515
ICU admission, surgical	47	29.7	30	30.6		17	28.3	0.761
ICU admission, trauma	9	5.7	4	4.1		5	8.3	0.302
Origin from other hospitals	34	21.5	23	23.5		11	18.3	0.446
CR infections presenting features								
VAP	65	41.1	35	35.7	30		50	0.076
HAP	20	12.6	12	12.2	8		13.3	0.842
BSI	54	34.2	35	35.7	19		31.7	0.603
cIAI	7	4.4	7	7.1	0		0	0.045
UTI	11	7	6	6.1	5		8.3	0.749
Other infections***	5	3.1	1	0.0001	4		6.6	0.069
ARF requiring MV	129	81.6	81	82.7	48		80.0	0.676
Septic Shock	43	27.2	28	28.5	15		25.0	0.624
Source control	46	29.1	39	39.7	7		11.6	< 0.002
Outcomes								
Treatment failure	78	49.3	49	50.0	29		48.3	0.839
Mortality in ICU	48	30.4	36	36.7	12		20	0.026

Categorical variables are expressed in count and percentage; continuous variables are expressed in median and interquartile range [IQR]

*Previous six months; **Including active neoplasm, chronic steroids, neutropenia, HIV with CD4 < 200/mmc, and immunosuppressive agents; ***Indicate Other infections

ABSSI, Acute Bacterial Skin and Soft Tissue Infection; *AKI*: Acute Kidney Injury; *ARF*: Acute Respiratory Failure; *BMI*. Body Mass Index; BLI, beta-lactams inhibitors; *BSI*, Bloodstream Infection; cIAI, complicated Intra-Abdominal Infection; *CR*, Carbapenem-resistant; *CRE*, Carbapenem-resistant Enterobacterales; *CRPA*, Carbapenem-resistant Pseudomonas aeruginosa; *CRRT*, Continuous Renal Replacement Therapy; *HAP*, Hospital-Acquired Pneumonia; *ICU*, Intensive Care Unit; *LOS*, Length Of Stay; *LTCF*, Long-Term Care Facility; *MV*, Mechanical Ventilation: *SOFA*, Sequential Organ Failure Assessment; *UTI*, Urinary-Tract Infection; *VAP*, Ventilator-Associated Pneumonia

the mortality in ICU rate and treatment failure rate were 37.5%.

The treatment success rate was 50.7% in the overall population, with 50.0% in patients with CRE and 51.7% in patients with CRPA. The cure rate through both clinical improvement and microbiological eradication (negative follow-up culture) was 32.3% overall, 32.7% in patients with CRE, and 31.7% in patients with CRPA. The cure rate through clinical improvement and suspected microbiological eradication (no follow-up culture) was 18.4% overall, 17.3% in patients with CRE, and 20.0% in patients with CRPA.

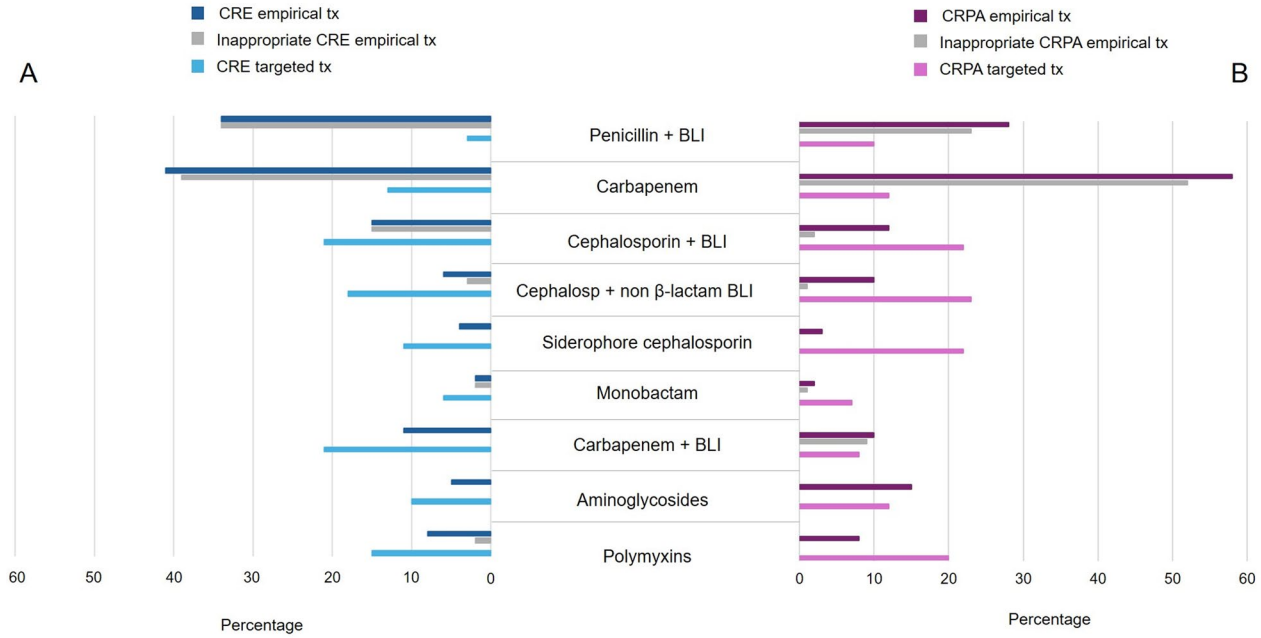

Fig. 2 Percentage of patients receiving classes of antibiotics against gram-negative bacteria as empirical, inappropriate empirical, and targeted therapy in A) CRE and B) CRPA groups

Table 2 Carbapenemases identified in 146 isolates of *Enterobacterales* and *Pseudomonas aeruginosa*

Microorganism (no. of strains)	Carbapenemase type(s) (no. of isolates)					
	KPC	NDM	OXA-48-like	VIM	KPC and OXA-48-like	KPC and VIM
Enterobacter cloacae complex (3)		NDM-1 (1)		VIM-1 (2)		
Escherichia coli (1)	KPC-2 (1)					
Klebsiella aerogenes (2)			OXA-181 (1)			
Klebsiella pneumoniae (87)	KPC-2 (5) KPC-3 (59) KPC-166 (6) KPC-167 (5) KPC-184 (1)	NDM-1 (6)		VIM-1 (1)	KPC-3 and OXA-181 (2)	KPC-2 and VIM-1 (1) KPC-3 and VIM-1 (1)
Providencia stuartii (1)		NDM-1 (1)				
Pseudomonas aeruginosa (52)				VIM-1 (1) VIM-2 (5)		

Discussion

This study confirmed the high burden of carbapenem-resistant strains in Italian ICUs compared to mean rates reported in Europe [10]. The overall prevalence of CRE and CRPA infections defined in 20 ICUs was 2.2% and 1.1%, respectively, while the prevalence of carbapenem resistance amongst total *Enterobacterales* and *P. aeruginosa* was 19.2% and 26.8%, respectively. These data confirm the rates recently reported by Scaglione and colleagues, who observed carbapenem-resistance rates of 21% in *Klebsiella* spp, and 25.3% in *P. aeruginosa* strains, respectively, across over 210 Italian ICUs in 2022 [11].

This prevalence was consistent with the Surveillance Report on AMR for 2023 by the European Centre for Disease Prevention and Control, which reported carbapenem-resistance rates of 26.5% in *Klebsiella* spp and 16% in *P. aeruginosa* strains from invasive isolates in different Italian wards [10]. Additionally, the epidemiological data from our study align with findings from the international EUROBACT-2 cohort study [12], which included 2600 patients with hospital-acquired bloodstream infections

Table 3 Antimicrobial susceptibility test results for 94 carbapenemase-producing *Enterobacterales*[a]

	MIC_{50}	MIC_{90}	MIC range	%S	%I	%R
Amoxicillin-clavulanic acid	≥ 32	≥ 32	≥ 32	0	0	100
Piperacillin-tazobactam	≥ 128	≥ 128	≥ 128	0	0	100
Cefotaxime	≥ 64	≥ 64	≥ 64	0	0	100
Ceftazidime	≥ 64	≥ 64	≥ 64	0	0	100
Ceftazidime-avibactam	2	≥ 64	0.5 to ≥ 64	74	0	26
Cefepime	≥ 16	≥ 16	≥ 16	0	0	100
Cefiderocol	–	–	–	91	0	9
Ceftolozane-tazobactam	≥ 64	≥ 64	≥ 64	0	0	100
Ertapenem	> 2	> 2	> 2	0	0	100
Imipenem	≥ 16	≥ 16	≤ 0.25 to ≥ 16	14	0	86
Imipenem-relebactam	0.25	≥ 32	≤ 0.25 to ≥ 32	83	0	17
Meropenem	≥ 64	≥ 64	≤ 0.12 to ≥ 64	14	2	84
Meropenem-vaborbactam	0.5	≥ 64	0.5 to ≥ 32	84	0	16
Aztreonam	≥ 32	≥ 32	≤ 1 to ≥ 32	0	6	94
Aztreonam-avibactam	0.25	0.5	0.03 to 1	100	0	0
Amikacin	≥ 32	≥ 32	≤ 4 to ≥ 32	46	0	54
Gentamicin	≥ 64	≥ 64	≤ 1 to ≥ 64	37	0	63
Ciprofloxacin	≥ 16	≥ 16	≤ 0.25 to ≥ 16	3	0	97
Colistin	0.5	≥ 16	≤ 0.25 to ≥ 16	74	0	26
Trimethoprim-sulfamethoxazole	≥ 8	≥ 8	≤ 1 to ≥ 8	33	0	67

[a] Includes *Enterobacter cloacae* complex (3). *Escherichia coli* (1). *Klebsiella aerogenes* (2). *Klebsiella pneumoniae* (87) and *Providencia stuartii* (1). MIC = minimum inhibitory concentration; % percentage. S: Susceptible, standard dosing regimen. I, Susceptible, increased exposure. R, Resistant

Table 4 Antimicrobial susceptibility testing results for 52 carbapenem-resistant *Pseudomonas aeruginosa*[a]

	MIC_{50}	MIC_{90}	MIC range	%S	%I	%R
Piperacillin-tazobactam	32	≥ 128	≤ 4 to ≥ 128	0	37	63
Ceftazidime	16	≥ 64	2 to ≥ 64	0	46	54
Ceftazidime-avibactam	2	≥ 64	1 to ≥ 64	85	0	15
Cefepime	8	≥ 16	1 to ≥ 16	0	54	46
Ceftolozane-tazobactam	1	≥ 64	≤ 0.5 to ≥ 64	85	0	15
Imipenem	≥ 16	≥ 16	8 to ≥ 16	0	0	100
Imipenem-relebactam	2	≥ 32	0.5 to ≥ 32	88	0	12
Meropenem	≥ 16	≥ 16	4 to ≥ 16	0	37	63
Meropenem-vaborbactam	≥ 64	≥ 64	4 to ≥ 64	37	0	63
Aztreonam	16	≥ 32	2 to ≥ 32	0	62	38
Cefiderocol	–	–	–	100	0	0
Amikacin	4	16	≤ 4 to ≥ 32	92	0	8
Ciprofloxacin	0.5	≥ 16	≤ 0.25 to ≥ 16	0	67	33
Colistin	1	1	≤ 0.5 to 2	100	0	0

[a] Includes isolates producing VIM-1 (n = 1) and VIM-2 (n = 5)

MIC Minimum inhibitory concentration; % percentage. S: Susceptible, standard dosing regimen. I, Susceptible, increased exposure. R, Resistant

(HA-BSI) from 333 ICUs across five continents. In that study, carbapenem resistance was observed in 37.8% of *Klebsiella* spp and 33.2% of *Pseudomonas* spp.

Carbapenem-resistance was associated with a high mortality rate in the ICU, affecting 30.4% of patients overall. Although the two cohorts differ, this data reflects the 28-day mortality rate observed in the EUROBACT-2 study (37.1%). Specifically, 91% of patients died in the ICU, while 9% died after ICU discharge. Interestingly, our study found that mortality was significantly higher in

patients with CRE (36.7%) compared to those with CRPA (20.0%). Additionally, the rate of inappropriate empirical therapy targeting gram-negative pathogens was notably high in the overall population (59.2%), particularly for CRE (74%) as opposed to CRPA (37.7%). This disparity in mortality and inappropriate therapy rates may be attributed to the inclusion criteria used. Specifically, carbapenemase production is just one of several resistance mechanisms employed by *P. aeruginosa*, whereas for *Enterobacterales*, it is considered the most concerning. Although with caution, it is also possible that the higher mortality in the CRE group was, at least in part, due to difference in clinical characteristics of the patients; for example, patients with CRE infections had a statistically significant higher number of individuals with a BMI over 30 kg/m^2 and a higher proportion of patients with renal impairment compared to the CRPA group; both of these factors can negatively affect the probability of attainment an adequate concentration of antibiotics, such as beta-lactams, at the infection site, thus reducing the probability of clinical efficacy [13].

Carbapenem-resistance was associated with high mortality rates in KPC and NDM-producing CRE, consistent with data from a recent observational study by the Italian ALARICO network [9]. In this study, the 30-day mortality rates were 26.5% for KPC-producing CRE and 36.4% for MBL-CRE. These findings underscored the importance of initiating appropriate antimicrobial therapy early to prevent unfavorable outcomes from CR-GNB infections.

Empirical therapy was inappropriate in 73.1% of KPC-producing CRE and 80% of MBL-producing isolates, indicating that significant selection pressure for KPC variants may have already been underway during the data collection period, as confirmed by our data. Unfortunately, several KPC variants drive different resistance patterns to currently prescribed beta-lactams plus BL inhibitors, thus reducing opportunities for an appropriate empirical therapy. These alarming rates of inappropriate empirical approaches may suggest a possible impact of these resistances on patient outcomes. However, this interpretation should be taken cautiously since our study did not analyse the independent association between inappropriate empirical therapy and mortality. Notably, this analysis has been recently performed in the EUROBACT-2 study resulting in a significant association between the adequacy of antimicrobial therapy within the first 24 h of HA-BSI and the decrease of 28-day mortality [14]. It's important to emphasize that these data, also supported by other studies [15], show the fundamental role of timing appropriateness in the early start of empirical therapy on mortality.

This study provides an important update on the in vitro susceptibility of CRE and CRPA strains to new antibiotics among Italian ICU patients. Although not yet available on the Italian market, AZA appears to be the most potent in vitro agent against all CRE isolates, showing promise as a valuable addition to the treatment arsenal, particularly against MBL-producing CRE. These results align with recent data on the in vitro activity of aztreonam-avibactam with respect to comparators, including the ARTEMIS study [16, 17]. Overall, the in vitro activity of ceftazidime-avibactam confirms that the drug continues to be a viable option for infections caused by not only CRE but also MDR *P. aeruginosa,* the latter suggested by the comparable results between ceftazidime-avibactam and ceftolozane-tazobactam also reported in International as well as Italian surveillance studies [18, 19]. The slightly reduced activity of ceftazidime-avibactam against CRE isolates is attributable to 11 isolates collected in two ICUs, suggesting the occurrence of two small clusters. These strains harboured two bla_{KPC} variants recently described in Italy, showing resistance to ceftazidime-avibactam and recovering susceptibility to imipenem and meropenem.

Interestingly, the 5 strains harbouring $bla_{\text{KPC-167}}$ were also resistant to a more recent antibiotic such as cefiderocol. Cluster occurrence aside, these data confirm an increase in ceftazidime-avibactam resistance observed in Italy over the past three years, though with significant inter-center variability, and warrant attention due to limited treatment options and the potential of further increase. That evidence highlights cautious prescription is required in areas or settings where resistant strains are known to circulate, suggesting that molecular rapid diagnostic and antibiotic susceptibility testing may be a key to identify these variants early to guide appropriate therapy.

Our study showed that the infectious disease specialist consultation was available daily in only 15.2% out of 20 ICUs and on-demand in 84.8% of ICUs. This may complicate the management of critically ill patients with severe infections, necessitating a multidisciplinary approach that enhances the surveillance of local epidemiology, guides the correct de-escalation/escalation strategies, optimizes antimicrobial dosing, and promotes effective source control. There are some critical limitations to be considered for this study. Notably, the study was conducted in a period ranging from June 2021 to February 2023 and, therefore, at least partially overlapped with the COVID-19 pandemic, which may have determined some discrepancies in patients' management across sites. Moreover, although infection prevention and control measures were implemented in participant sites, the COVID-19 pandemic may have

contributed to the increase in the number of hospital-acquired infections as well as to the high rates of infections caused by multi-drug-resistant bacteria in Italian ICUs. In addition, even if the 20 participant sites were well distributed along the Italian territory, we cannot exclude a certain degree of non-homogeneity; therefore, the findings of this study cannot be generalizable to the overall cohort of ICUs in Italy. Finally, we recognize that an important limitation of the study is the unavailability of analysing factors independently associated with treatment failure and mortality that does not allow us to make conclusions on the association between high rate of inappropriate empirical therapy and patient outcomes. Despite the above limitations, findings of this observational study have shown further evidence on the burden of carbapenem-resistance in Italian ICUs, pointing out the spread of emerging variants and providing up-to-date data on the in vitro susceptibility of CRE and CRPA strains to old and new antibiotics. Implementation of infection prevention and control measures, risk stratification and application of molecular rapid diagnostic and antibiotic susceptibility testing can contribute to improve patient management.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13054-025-05266-1.

Additional file 1 .

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Author contributions
All authors conceived the study, collected and analysed data, wrote, edited and revised critically the manuscript. All authors approved current version for submission.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. Data is provided within the manuscript or supplementary information files.

Declarations

Consent for publication
Not applicable.

Competing interests
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Author details
[1]Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Rome, Italy. [2]Dipartimento di Scienze Dell'Emergenza, Anestesiologiche e Della Rianimazione, Fondazione Policlinico Universitario A. Gemelli IRCCS, Cattolica del Sacro Cuore, Rome, Italy. [3]Department of Anesthesia, Analgesia, Intensive Care and Emergency, University Hospital Policlinico Paolo Giaccone, Palermo, Italy. [4]Department of Precision Medicine in Medical, Surgical and Critical Care (Me.Pre.C.C.), University of Palermo, Palermo, Italy. [5]Department of Medical and Surgical Sciences, Alma Mater Studiorum, University of Bologna, Bologna, Italy. [6]Infectious Disease Unit, Department for Integrated Risk Management, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy. [7]Division of Cardiothoracic Intensive Care, ASST Spedali Civili, Brescia, Italy. [8]Department of Anesthesia and Intensive Care Medicine, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy. [9]Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Milan, Italy. [10]Department of Anesthesia and Intensive Care, IRCCS San Raffaele Scientific Institute, Milan, Italy. [11]Intensive Care Unit II, Department of Precision Medicine, Ionic Area, University of Bari "A. Moro", Bari, Italy. [12]Unità Operativa Complessa Rianimazione DEA, Ospedale Cardarelli, Naples, Italy. [13]Department of Biomedical Sciences and Public Health, Università Politecnica Delle Marche, Ancona, Italy. [14]Anesthesia and Intensive Care, Azienda Ospedaliero Universitaria Delle Marche, Ancona, Italy. [15]Medical Affairs, Pfizer Italia, Rome, Italy. [16]Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialities, University of Palermo, Palermo, Italy. [17]School of Medicine, University of Milan Bicocca, Monza, Italy. [18]IRCCS San Gerardo dei Tintori Foundation, Monza, Italy. [19]Unit of Intensive Care, Pescara General Hospital, Pescara, Italy. [20]Department of Medicine and Surgery, University of Milan-Bicocca, Monza, Italy. [21]Department of Anesthesia and Intensive Care Medicine, Niguarda Ca' Granda, Milan, Italy. [22]Unit of Anesthesiology and Intensive Care Unit, University Hospital of Modena, University of Modena and Reggio Emilia, Modena, Italy. [23]Division of Cardiac Surgery, Department of Surgery, Dentistry, Pediatrics and Gynecology, University of Verona, Verona, Italy. [24]Operative Research Unit of Anesthesia and Intensive Care, Campus Bio-Medico University Hospital, Rome, Italy. [25]Department of Surgical Sciences, University of Turin, Turin, Italy. [26]Department of Anesthesia, Intensive Care and Emergency, Città della Salute e della Scienza University Hospital, Turin, Italy. [27]Department of Medicine, Surgery and Pharmacy, University of Sassari, Sassari, Italy. [28]Anesthesia and Intensive Care Unit, Civil Hospital of Alghero, Alghero, Sassari, Italy. [29]Department of Anesthesiology, Neuroanesthesia and Intensive Care Unit, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy. [30]Department of Critical Care Medicine and Anesthesiology, St. Maria Hospital, Terni, Italy. [31]Department of Neurosciences, Reproductive and Odontostomatological Sciences, University of Naples "Federico II", Naples, Italy. [32]Dipartimento di Scienze di Laboratorio ed Ematologiche, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy.

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