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High rate of colistin resistance among patients with carbapenem-resistant *Klebsiella pneumoniae* infection accounts for an excess of mortality

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Abstract

Carbapenem-resistant *Klebsiella pneumoniae* (CR-KP) is becoming a common cause of healthcare-associated infection in Italy, with high morbidity and mortality. Prevalent CR-KP clones and resistance mechanisms vary between regions and over time. Therapeutic approaches and their impact on mortality have to be investigated. We performed a prospective study of patients with CR-KP isolation, hospitalized in nine hospitals of Rome, Italy, from December 2010 to May 2011, to describe the molecular epidemiology, antibiotic treatment and risk factors for mortality. Overall, 97 patients (60% male, median age 69 years) were enrolled. Strains producing *bla*KPC-3 were identified in 89 patients, *bla*VIM in three patients and *bla*CTX-M-15 plus porin defects in the remaining five patients. Inter-hospital spread of two major clones, ST512 and ST258, was found. Overall, 36.1% and 20.4% of strains were also resistant to colistin and tigecycline, respectively. Infection was diagnosed in 91 patients who received appropriate antibiotic treatment, combination therapy and removal of the infectious source in 73.6%, 59.3% and 28.5% of cases, respectively. Overall, 23 different antibiotic regimens were prescribed. In-hospital mortality was 25.8%. Multivariate analysis adjusted for appropriate treatment, combination therapy and infectious-source removal, showed that Charlson comorbidity score, intensive-care unit onset of infection, bacteraemia and infection due to a colistin-resistant CR-KP strain were independent risk factors for mortality. The spread of clones producing *K. pneumoniae* carbapenemases, mainly ST258, is currently the major cause of CR-KP infection in central Italy. We observed a high rate of resistance to colistin that is independently associated with worse outcome.

Keywords: Carbapenem resistance, carbapenemase, colistin resistance, in-hospital mortality, *Klebsiella pneumoniae*
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Introduction

Carbapenem resistance represents the current challenge in the treatment of infections caused by Gram-negative bacteria [1]. In Italy, since the first detections of resistance to carbapenems in *Acinetobacter baumannii* and *Pseudomonas aeruginosa* during the late 1990s [2,3], concern has risen for the detection of

carbapenem-resistant Enterobacteriaceae, especially *Klebsiella pneumoniae* [4–7].

Carbapenem resistance can be the result of various mechanisms including the production of a carbapenemase enzyme, such as metallo-β-lactamases, *K. pneumoniae* carbapenemases (KPCs), and OXA-48; and the combination of porins defect plus extended spectrum β-lactamase (ESBL) or AmpC enzyme production [8]. Strains producing carbapenemases are more resistant to carbapenems compared with those with other mechanisms of resistance, and their genes may be achieved horizontally, allowing them to spread widely. In Italy, carbapenem-resistant *K. pneumoniae* (CR-KP) strains showing the combination of porins defect plus ESBL production were becoming common in some centres [9], whereas in others carbapenemase-producing strains were prevalent and, in most cases, they were clonally related [10].

The therapeutic armamentarium against infection by CR-KP is limited to antibiotics with high potential toxicity, such as colistin and gentamicin, or with a poor pharmacokinetic/pharmacodynamic profile, as tigecycline. The use of these antibiotics has been associated with the emergence of resistance against them [11]. For these reasons, most authors recommend using combination therapy with different classes of antibiotics to improve the efficacy and to prevent the emergence of further resistance [12]. However, the evidence about which combination is optimal is far from clear, and the impact of resistance to the last antimicrobial choices available in clinical practice is unknown.

We performed a prospective multicentre study in nine hospitals in Rome, Italy, to assess which are the current prevalent clones and mechanisms of resistance among CR-KP isolates, to describe the characteristics of patients colonized or infected by such strains, and to analyse the therapeutic management of patients with CR-KP infection and the predictors of mortality in these patients.

Methods

Study design and setting

We performed a multicentre prospective observational study based on an alert system provided by nine microbiology laboratories belonging to the SEERBIO-GRAB network. These laboratories serve a population of *c.* 2.5 million people in Rome, and analyse clinical samples collected in one teaching institution, six tertiary hospitals, one clinical and research institute, and one long-term care facility, with a total of 4000 beds, ranging from 100 to 1200 beds per centre.

During December 2010 through to May 2011, the clinical and microbiological data of all consecutive patients with isolation of a *K. pneumoniae* strain showing reduced susceptibility to ertapenem (MIC $\geq$ 1 mg/L), from different specimens, were collected. An investigator contacted the participating centres to monitor the inclusion of all eligible cases. The completeness and consistency of the protocols were systematically reviewed before data were entered into the database.

Diagnostic and therapeutic management for all patients, including the need to obtain surveillance cultures, was not standardized and decisions were made at the discretion of the attending physician.

The study involved the analysis of existing clinical and laboratory data that were anonymous (an alphanumeric code, composed of a letter identifying the hospital of origin and the number indicating the clinical history, was assigned to each patient) before being entered in the database. Hence, according to local and national regulations, this analysis was exempt from formal approval by the Ethics Committee.

Clinical data and definitions

The medical charts of the patients were reviewed according to a pre-established protocol including the following variables: age, sex, diabetes, chronic obstructive pulmonary disease, renal insufficiency (with or without dialysis), liver cirrhosis, active cancer, human immunodeficiency virus infection, solid organ transplantation, haematological disease with or without stem cell transplantation, and corticosteroid therapy ($\geq$ 10 mg/day of prednisone during $\geq$ 15 days).

Data on prior healthcare exposure included: admission from other hospital or long-term care facility, previous hospitalization in the past 12 months, prior admission to intensive-care unit (ICU) or major surgery in the past 30 days, and receipt of any antibiotic during $\geq$ 48 h in the past 30 days.

Dates of hospital admission and of CR-KP isolation, ward of stay, and presence of devices including central venous catheter (CVC), mechanical ventilation and urinary catheter at the time of CR-KP isolation were recorded.

Infection was established according to standard definitions [13], the probable infectious source was determined on the basis of the microbiological results and physician's judgment. Bloodstream infections (BSI) were defined as low-risk BSI if the portal of entry was the CVC or urinary tract, and as high-risk BSI in the other cases [14].

Data on therapeutic management included: type, dosage, route of administration and dates of start and of end for any antibiotic. Appropriate antimicrobial therapy was defined as treatment with at least one *in vitro* active antibiotic for a minimum of 48 h [15]. Removal of the infectious source was also recorded.

Outcome was evaluated using the following: development of septic shock; in-hospital death; and length of hospitalization from the CR-KP isolation.

Strain identification and antimicrobial susceptibility test

Isolates were studied at the participating centres and sent to two reference laboratories (Istituto Superiore di Sanità and San Filippo Neri).

Klebsiella pneumoniae identification and antimicrobial susceptibility were determined by individual laboratories using the Vitek2 system, AST-N089 card (bioMérieux, Marcy l'Etoile, France).

Ertapenem-resistant strains (MIC >1 mg/L) were sent to Istituto Superiore di Sanità, Rome, for confirmation and molecular screening of resistance mechanisms. All strains were tested for carbapenemase production by modified Hodge test [16]. The carbapenemase-positive strains were tested by combined-disc method using a disc of meropenem (10 μg) with or without 400 μg phenylboronic acid or 10 μL 0.1 M EDTA on Mueller–Hinton agar II [17].

Antibiotic susceptibilities to carbapenems, colistin, tigecycline, aminoglycosides and fosfomycin were confirmed by the San Filippo Neri laboratory using the microdilution method. Breakpoints were interpreted in accordance with the European Committee on Antimicrobial Susceptibility Testing guidelines.

β-Lactamase gene detection and characterization of porin genes

All the ertapenem-resistant/Hodge test-positive strains were tested by PCR amplification and DNA sequencing for the presence of the bla_{KPC}, bla_{VIM}, bla_{NDM}, bla_{OXA-48}, bla_{CTX-M} and bla_{CMY} genes, using methodology and primers that were described previously [18–20]. Full-length sequences were obtained for the bla_{CTX-M}, *ompK35* and *ompK36* genes in the ertapenem-resistant/Hodge test-negative strains, using previously described methodology and primers [21].

Strain genotyping

The *gapA* and *tonB* genes, chosen as the most discriminatory alleles of the multilocus sequence typing were initially amplified and sequenced to categorize the strains in four major groups: ST258-like (*gapA* allele 3, *tonB* allele 79), ST512-like (*gapA* allele 54, *tonB* allele 79), ST37-like (*gapA* allele 2, *tonB* allele 16) and other sequence types (other-STs), respectively. Fifty-five selected strains (16 ST258-like, 20 ST512-like, four ST-37 and eight strains showing other-STs) were fully typed by multilocus sequence typing performed on the *rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB* and *tonB* genes, as previously described [22]. Sequence types were assigned at the http://www.pasteur.fr/recherche/genopole/PF8/mlst/ website. Plasmid typing was performed by PCR-based replicon typing as previously described [23,24].

Statistical analysis

Categorical variables are presented as absolute numbers and their relative frequencies. Quantitative variables are presented as mean and standard deviation (SD) if normally distributed or as median and interquartile range (IQR) if non-normally distributed. We compared categorical variables between two groups (survivors and non-survivors) using the Pearson chi-square and Fisher exact tests, while the parametric Student's *t* or non-parametric Mann–Whitney *U* tests were used to compare quantitative variables, depending on their distribution. A binary logistic regression model was used in the multivariate analysis to analyse risk factors for in-hospital mortality. Variables with $p < 0.1$ in the univariate analysis were included in the multivariate models. Differences were considered to be significant for $p < 0.05$. The analysis was carried out using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

Results

Microbiology characteristics

During the study period, 97 patients with isolation of a CR-KP strain from one or more samples including: urine (34), blood (34), lower respiratory tract (13), surgical wound (8), intra-abdominal fluid (7), CVC tips (12), rectal swab (3) and cerebrospinal fluid (1) were identified (Fig. A1 in Appendix 2).

The rate of antimicrobial resistance was 95.9%, 89.8%, 79.6%, 36.1% and 20.4% for imipenem, meropenem, gentamicin, colistin and tigecycline, respectively. Susceptibility testing to fosfomycin was performed for 24 strains, 12 (50%) of them were found to be resistant. The overall distribution of the colistin MIC values is shown in Table A1 (in Appendix 2).

The following mechanisms of carbapenem resistance were identified: production of KPC-3 in 89 cases; production of VIM-1 in three cases (all from the same hospital); and production of CTX-M-15 plus porin defects in the remaining five patients. The modified Hodge test was positive for all strains producing carbapenemases, but was negative for those producing ESBLs associated with porin defect.

Among strains producing KPC-3, two major clones were identified by multilocus sequence typing: ST512 and ST258, belonging to the same clonal complex (CC-258). KPC-3 was also identified in clones ST646 (new ST), ST650 (new ST), ST14 and ST101 (Table 1). The bla_{VIM-1} gene was identified in clones ST646, ST647 and ST648 (three new STs). Among strains producing ESBL combined with outer membrane protein (OmpK) defects, three belonged to ST37, and the other was assigned to the new ST649 (Table 1).

The bla_{KPC-3} gene was located on plasmids carrying the FIIk and FIBk replicons. The same replicon content was observed in strains ST512, ST258, ST650, ST14 and ST101. The ST258 clones identified in 14 strains from two hospitals harboured an additional IncA/C plasmid, carrying the CMY-2 AmpC β-lactamase.

TABLE 1. Molecular typing results and β-lactamases genes in *Klebsiella pneumoniae* analysed in this study

No. strains	Hospitals	CARB. gene	CTX	CMY	PBRT	MLST
71	A, B, C, D, E, F	bla_{KPC-3}	neg	neg	FIIk, FIBk	ST512, ST258 (CC-258)
14	B, G	bla_{KPC-3}	neg	bla_{CMY-2}	FIIk, FIBk, A/C	ST258
1	B	bla_{KPC-3}	neg	neg	FIIk, FIBk	ST650
1	H	bla_{KPC-3}	neg	neg	FIIk, FIBk	ST14
1	E	bla_{KPC-3}	neg	neg	FIIk, FIBk	ST101
1	E	bla_{KPC-3}	neg	neg	FIIk, N	ST646
1	E	bla_{VIM-1}	neg	neg	FIIk, N	ST646
1	E	bla_{VIM-1}	neg	neg	FIIk, A/C	ST648
1	E	bla_{VIM-1}	neg	neg	FIIk	ST647
4	A, B	neg	$bla_{CTX-M-15}$	neg	FIIk, FII	ST37
1	I	neg	$bla_{CTX-M-15}$	neg	FIIk, FII	ST649

CARB, carbapenemase; PBRT, PCR-based replicon typing [20]; MLST, multi-locus sequence typing [22].

TABLE 2. Characteristics of patients colonized or infected by carbapenem-resistant *Klebsiella pneumoniae* (CR-KP)

	(*n* = 97), *n* (%)
Demographic data	
Age (years, median, IQR)	69, 50–77
Male sex	60 (61.9)
Underlying diseases	
Immunosuppression[a]	43 (44.3)
Diabetes	34 (35)
Chronic obstructive pulmonary disease	33 (34)
Chronic kidney disease	28 (28.9)
Cancer	20 (20.6)
Chronic liver disease	6 (6.2)
Charlson comorbidity score (median, IQR)	5, 3–8
Prior healthcare exposure	
Hospitalization in the past 1 year	89 (91.8)
Admission to intensive-care unit in the past 1 month	74 (76.3)
Surgery in the past 1 month	68 (70)
Antibiotic exposure in the past 30 days	
Any	95 (97.9)
β-Lactams plus β-lactamase inhibitors	53 (54.6)
Fluoroquinolones	45 (46.9)
Carbapenems	40 (41.7)
Glycopeptides	23 (23.7)
Third-generation cephalosporins	20 (20.8)
Linezolid	16 (16.7)
Metronidazole	8 (8.2)
Colistin	6 (6.2)
Aminoglycosides	5 (5.2)
Tigecycline	4 (4)
Ward, length of stay and devices at the time of CR-KP isolation	
Intensive-care unit	46 (47.4)
Medical	37 (38.1)
Surgical	14 (14.4)
Days of hospitalization before CR-KP isolation (median, IQR)	15, 8–32
Urinary catheter	90 (92.8)
Central venous catheter	67 (69)
Mechanical ventilation	46 (47.4)
APACHE II score (median, IQR)[b]	15, 12–20
Clinical syndrome	
Colonization	6 (6.2)
Urinary tract infection	29 (29.9)
Bloodstream infection (BSI)	34 (35.1)
Low risk BSI	16 (16.5)
High risk BSI	18 (18.6)
Lower respiratory tract infection	14 (14.4)
Skin and soft tissues infection[c]	11 (11.3)
Intra-abdominal infection	3 (3.1)
Outcome	
Septic shock	15 (15.2)
In-hospital death	25 (25.8)
Length of hospital stay[d] (days, median, IQR)	20, 12–33

IQR, interquartile range; ICU, intensive care unit.
[a]Immunosuppression includes patients with solid organ transplantation, corticosteroid therapy, and human immunodeficiency virus infection.
[b]APACHE II score at the ICU admission was calculated for the 46 patients hospitalized in ICU at the time of CR-KP isolation.
[c]Skin and soft tissues infection includes surgical site infections.
[d]Length of hospital stay was computed since the isolation of CR-KP.

Clinical characteristics

The clinical characteristics of the patients are summarized in Table 2.

The median age was 69 years (IQR 50–77). Most patients had previous healthcare exposure, and had received antibiotics in the last 30 days. At the time of CR-KP isolation, patients were hospitalized in ICU, medical and surgical wards in 47.4%, 38.1% and 14.4% of cases, respectively.

Infection was diagnosed in 91 patients and colonization was established in the remaining six patients (Table 2).

The 35 CR-KP strains that were resistant to colistin caused infection in 32 patients (including twelve with BSI; nine with uncomplicated urinary infection; five with lower respiratory tract infection; five with surgical site infection; and one with an intra-abdominal infection) and were deemed as colonizing in the remaining three cases.

The overall in-hospital mortality among CR-KP-infected patients was 25.8%. The mortality rates among patients infected by colistin-susceptible and colistin-resistant CR-KP were 20.3% and 40.6% (p 0.04), respectively.

Therapeutic management

The antimicrobial treatment was deemed appropriate in 73.6% of patients with CR-KP infection. Monotherapy and combination therapy, with two or more classes of antibiotics, were administered in 40.7% and 59.3% of cases, respectively. The antibiotics used were: colistin, 29.6%; tigecycline, 24.6%; aminoglycosides, 21.6%; fosfomycin, 13.5%; piperacillin-tazobactam, 4.3%; carbapenems, 3%; and third-generation cephalosporins, 3%.

Overall, 23 different regimens were prescribed, the most common were: monotherapy with gentamicin, 17.6%; combination therapy with colistin plus tigecycline, 17.6%; monotherapy with colistin, 11%; and combination therapy with tigecycline plus fosfomycin, 6.6%. Ten patients (11%) received combination therapy with three different classes of antibiotics.

Overall, 48 patients received colistin for CR-KP infection, 11 of them as monotherapy and 37 in combination with other agents. The relationship between colistin MIC values and mortality for both groups was analysed (Table A2 in Appendix 2). Patients with isolates showing lower colistin MIC values had better outcomes.

Fourteen of the 16 patients treated with gentamicin monotherapy had uncomplicated urinary infection; the other two presented with bacteraemia from urinary source and one of them died on septic shock during treatment.

Removal of the infectious source was performed in 26 patients, in 14 by surgical drainage, and in 12 (all with CVC-related BSI) by removal of the CVC.

Risk factors for in-hospital mortality

Univariate analysis of the factors associated with in-hospital mortality is shown in Table 3. Multivariate analysis adjusted for appropriate antibiotic treatment, combination therapy, and removal of the infectious source, showed that the Charlson comorbidity score (OR 1.42, 95% CI 1.15–1.76, p 0.001); ICU onset of the CR-KP infection (OR 18.05, 95% CI 3.90–83.51, p <0.001); BSI (OR 4.92, 95% CI 1.35–17.28, p 0.01); and infection caused by a colistin-resistant strain (OR 4.15, 95% CI 1.17–14.74, p 0.02) were the independent risk factors for in-hospital mortality (Table 4).

A subset analysis of risk factors for in-hospital mortality among patients with CR-KP BSI showed that age, chronic kidney disease, Charlson comorbidity score and ICU onset of CR-KP BSI were the variables significantly associated with mortality (Table 5). Multivariate analysis adjusted for appropriate antibiotic treatment, combination therapy and removal of the infectious source, showed Charlson comorbidity score (OR 1.63, 95% CI 1.12–2.35, p 0.008) and ICU onset of CR-KP infection (OR 31.94, 95% CI 2.43–419.44, p 0.009) to be the independent risk factors for in-hospital mortality in this subgroup.

TABLE 3. Univariate analysis of risk factors for in-hospital mortality among 91 patients infected by carbapenem-resistant *Klebsiella pneumoniae* (CR-KP)

	Survivors (*n* = 66), *n* (%)	Non-survivors (*n* = 25), *n* (%)	p
Demographic data			
Age (years, median, IQR)	68, 45.7–75.2	75, 60–77.5	0.05
Male sex	40 (60.6)	15 (60)	1
Underlying conditions			
Immunosuppression[a]	27 (40.9)	15 (60)	0.15
Diabetes	23 (34.8)	8 (32)	0.81
Chronic obstructive pulmonary disease	18 (27.3)	13 (52)	0.04
Chronic kidney disease	15 (22.7)	12 (48)	0.02
Cancer	13 (19.7)	6 (24)	0.77
Chronic liver disease	4 (6.1)	2 (8)	1
Charlson score (median, IQR)	5, 2–8	6, 4–10	0.03
Days of stay before isolation (median, IQR)	12.5, 7–37	17, 13–25	0.29
Ward of hospitalization			
Intensive-care unit	23 (34.8)	21 (84)	<0.001
APACHE II score (median, IQR)[b]	14, 12–17	18, 12–22	0.12
Medical	34 (51.5)	2 (8)	<0.001
Surgical	9 (13.6)	2 (8)	0.51
Mechanism of carbapenem resistance			
K. pneumoniae carbapenemases	59 (89.4)	24 (96)	0.69
Verona integron-encoded metallo-β-lactamase	3 (4.5)	0	
Extended spectrum β-lactamases + OmpKs	4 (6.1)	1 (4)	
Antibiotic resistance			
Imipenem	64 (97)	24 (96)	1
Meropenem	57 (86.4)	24 (96)	0.27
Gentamicin	51 (77.3)	21 (84)	0.57
Colistin	19 (28.8)	13 (52)	0.05
Tigecycline	15 (22.7)	2 (8)	0.14
Fosfomycin	8/14 (57.1)	3/9 (33.3)	0.40
Type of infection			
Urinary tract infection	29 (43.9)	0	<0.001
Bloodstream infection (BSI)	18 (27.3)	16 (64)	0.002
Low-risk BSI	10 (15.2)	6 (24)	0.36
High-risk BSI	8 (12.1)	10 (40)	0.006
Lower respiratory tract infection	8 (12.1)	6 (24)	0.19
Skin and soft tissues infection[c]	9 (13.6)	2 (8)	1
Intra-abdominal infection	2 (3)	1 (4)	1
Septic shock	0	15 (60)	<0.001
Therapeutic management			
Appropriate antibiotic therapy	50 (75.8)	17 (68)	0.59
Antibiotic therapy with two or more antibiotics	37 (56.1)	17 (68)	0.34
Gentamicin monotherapy	15 (22.7)	1 (4)	0.03
Colistin monotherapy	6 (9.1)	4 (16)	0.45
Colistin plus tigecycline	12 (18.2)	4 (16)	1
Colistin plus fosfomycin	5 (7.6)	0	0.32
Colistin plus gentamicin	3 (4.5)	2 (8)	0.61
Tigecycline plus fosfomycin	4 (6.1)	2 (8)	1
Removal of the infectious source	19 (28)	7 (28)	1

IQR, interquartile range; ICU, intensive care unit; OmpKs, outer membrane proteins.
[a]Immunosuppression includes patients with solid organ transplantation, corticosteroid therapy, and human immunodeficiency virus infection.
[b]APACHE II score at the ICU admission was calculated for the 46 patients hospitalized in ICU at the time of CR-KP isolation.
[c]Skin and soft tissues infection includes surgical site infections.

Discussion

Our findings are consistent with the spread of KPC-3-producing strains in the hospitals of our region. One-third of the strains were also resistant to colistin. Combination therapy was the treatment of choice in *c.* 60% of CR-KP infections, with a wide heterogeneity in the antibiotics combination used. Overall, in-hospital mortality was 25.8%. Underlying conditions, bacteraemia and resistance to colistin, were the independent risk factors for mortality.

In the last years, *K. pneumoniae* CC258 producing the KPC-carbapenemase has been detected as one of the most important nosocomial pathogens worldwide [1]. We confirmed these data, indeed 90% of the CR-KP collected in our study were found to be KPC-producing strains belonging to the CC258 clone. Furthermore, we observed both the carriage of similar KPC-harbouring plasmids within genetically different strains and the inter-hospital and intra-hospital spread of ST258 and ST512 strains belonging to the CC258 clone.

In our study, the *blaKPC-3* gene has been found on pKpQIL-related plasmids. These plasmids are characterized by the

TABLE 4. Multivariate analysis of risk factors for in-hospital mortality in patients with infection due carbapenem-resistant *Klebsiella pneumoniae* (CR-KP), adjusted for appropriate antibiotic treatment, combination therapy and removal of the infectious source

	OR (95% CI)	p
Charlson comorbidity score	1.42 (1.15–1.76)	0.001
Hospitalization in intensive-care unit	18.05 (3.90–83.51)	<0.001
Bloodstream infection	4.92 (1.35–17.28)	0.01
Infection due to a colistin-resistant strain	4.15 (1.17–14.74)	0.02

TABLE 5. Univariate analysis of risk factors for in-hospital mortality among patients with carbapenem-resistant *Klebsiella pneumoniae* bloodstream infection

	Survivors (*n* = 18), *n* (%)	Non-survivors (*n* = 16), *n* (%)	p
Demographic data			
Age (years, median, IQR)	54.5, 39–70.2	72, 58.2–77	0.02
Male sex	12 (66.7)	8 (50)	0.48
Underlying conditions			
Immunosuppression[a]	8 (44.4)	11 (68.8)	0.18
Diabetes	7 (38.9)	7 (43.8)	1
Chronic obstructive pulmonary disease	4 (22.2)	7 (43.8)	0.27
Chronic kidney disease	2 (11.1)	10 (62.5)	0.003
Cancer	4 (22.2)	3 (18.8)	1
Chronic liver disease	0	2 (12.5)	0.21
Charlson score (median, IQR)	3.5, 1–6	6, 4–10.7	0.03
Days of stay before isolation (median, IQR)	12, 6.7–26.7	18, 11.7–31	0.25
Ward of hospitalization			
Intensive-care unit	9 (50)	14 (87.5)	0.03
APACHE II score (median, IQR)[b]	14, 12–19	20, 13–22	0.22
Medical	5 (27.8)	2 (12.5)	0.40
Surgical	4 (22.2)	0	0.10
Mechanism of carbapenem resistance			
K. pneumoniae carbapenemases	16 (88.9)	15 (93.8)	1
Enhanced spectrum β-lactamases + OmpKs	2 (11.1)	1 (6.2)	
Colistin resistance	5 (27.8)	7 (43.8)	0.47
Type of bloodstream infection (BSI)			
Low-risk BSI	10 (55.6)	6 (37.5)	0.32
High-risk BSI	8 (44.4)	10 (62.5)	
Septic shock	0	11 (68.8)	<0.001
Therapeutic management			
Appropriate antibiotic therapy	16 (88.9)	12 (75)	0.38
Antibiotic therapy with at least two antibiotics	14 (77.8)	11 (68.8)	0.70
Removal of the infectious source	1 (5.6)	2 (12.5)	0.59

IQR, interquartile range; OmpKs, outer membrane proteins.
[a]Immunosuppression includes patients with solid organ transplantation, corticosteroid therapy, and human immunodeficiency virus infection.
[b]APACHE II score at the ICU admission was calculated for the 23 patients hospitalized in ICU at the time of CR-KP BSI onset.

presence of two replicons, namely FIIk and FIBk, and have been previously reported and fully sequenced in *K. pneumoniae* ST258 from Israel, and in the first *K. pneumoniae* ST258 case to occur in one of the hospitals of our study in 2010 [25,26]. In this latter hospital, in which surveillance data were available before the beginning of our study, we were also able to show a shift from the ST37 clone, showing ESBL production plus a porins defect, to the KPC-3 ST512 clone, rapidly becoming the most prevalent lineage detected among CR-KP [9].

Management of CR-KP infections presents major challenges. In the literature, clinical data are very limited and consist mainly of small case series and brief reports [27,28]. In a systematic review [27], combination treatment with aminoglycosides (75%), polymyxin (73%) and tigecycline (71%) had higher success rates than monotherapy with carbapenem (40%) or polymyxin (14%). Indeed, some experts recommend the use of combination therapy for treatment of severe CR-KP infections [27,28]; this recommendation was fulfilled in about 60% of cases in our study.

The in-hospital mortality rate reported in this study among patients with CR-KP infection was lower than in previous reports [15,29,30]. However, as previously showed by other authors [31], we found that the rate of mortality rose to 47% among the 34 patients with bacteraemia and BSI was one of the independent risk factors for in-hospital mortality.

We found an unexpectedly high rate of colistin resistance among our strains. Maybe the increased use of this drug during recent years, especially as monotherapy, could be the cause of this. Unfortunately, data on colistin use in the participating hospitals, previously and during the study period, were not available. Furthermore, infection due to a colistin-resistant CR-KP was an independent risk factor for mortality. Resistance to colistin could be simply a marker of the patient's severity as shown in infections caused by other resistant microorganisms such as *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* [14,32]. However, physicians should be aware of such an occurrence for its implications on treatment and outcome.

Our study has some limitations. The small number of cases reduces the power of the analysis of risk factors for mortality as well as the heterogeneity in the infections studied and antimicrobial treatments.

In conclusion, we showed that the CC258 KPC-producing strain is becoming prevalent in many hospitals of our region, mainly due to the inter-hospital and intra-hospital spread of two major clones. We confirmed the high level of healthcare exposure among patients colonized or infected by CR-KP. We found a high rate of resistance to colistin and a high heterogeneity in the antimicrobial approach. The underlying conditions, type of CR-KP infection and infection due to a colistin-resistant strain were independently associated with worse outcome.

Competing Interests

Alessandro Capone, Maddalena Giannella, Daniela Fortini, Alessandra Giordano, Marcello Meledandri, Milva Ballardini, Mario Venditti, Daniela Capozzi, Maria Pia Balice, Agapito

Tarasi, Gabriella Parisi, Angelo Lappa, Alessandra Carattoli have no conflicts of interest. Nicola Petrosillo received honoraria as speaker for Pfizer, Astellas, Sanofi Aventis, Wyeth, Glaxo SmithKline, Merk Sharp & Dohme, Novartis, Carefusion, Johnson & Johnson, Janssen Cilag and Bristol Myers Squibb.

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Transparency Declaration

This study does not present any conflict of interests for the authors.

Appendix 1

Members of the SEERBIO-GRAB network

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Appendix 2

Fig. A1. Number of cases provided by each laboratory/hospital.

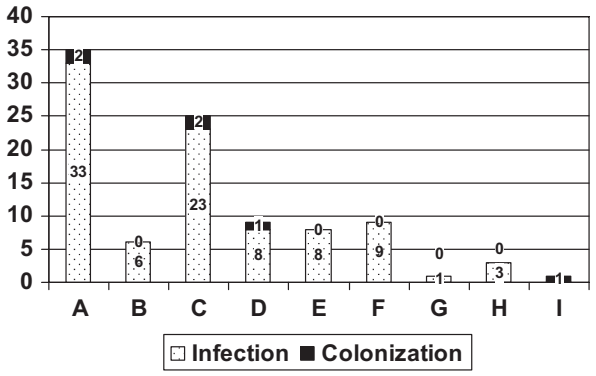

Table A1. Distribution of the colistin MICs

MIC value (mg/L)	Strains from colonized patients *n* = 6 *n* (%)	Strains from infected patients *n* = 91, *n* (%)	Total *n* = 97, *n* (%)
0.38	0	1 (1.1)	1 (1)
0.50	3 (50)	58 (63.7)	61 (62.9)
1.00	0	1 (1.1)	1 (1)
2.00	0	1 (1.1)	1 (1)
8.00	0	1 (1.1)	1 (1)
16.00	3 (50)	29 (31.9)	32 (33)

Table A2. Relationship between colistin MIC values and outcome among patients with carbapenem-resistant *Klebsiella pneumoniae* (CR-KP) infection treated with colistin as monotherapy or combination therapy

Monotherapy	Survivors *n* = 7, *n* (%)	Non-survivors *n* = 4, *n* (%)	p
Colistin MIC values			
0.50	6 (85.7)	2 (50)	0.11
2	1 (14.3)	0	
16	0	2 (50)	
Combination therapy	*n* = 25, *n* (%)	*n* = 12, *n* (%)	
Colistin MIC values			
0.50	20 (80)	6 (50)	0.05
1	1 (4)	0	
16	4 (16)	6 (50)	

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