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External validation of the INCREMENT-CPE mortality score in a carbapenem-resistant *Klebsiella pneumoniae* bacteraemia cohort: the prognostic significance of colistin resistance

Isabel Machuca[a,1,2], Belén Gutiérrez-Gutiérrez[b,1,2], Francisco Rivera-Espinar[c], Angela Cano[a,2], Irene Gracia-Ahufinger[d,2], Julia Guzman-Puche[d], Eduardo Marfil-Pérez[d], Elena Pérez-Nadales[a,2], Juan José Castón[a,2], Robert A. Bonomo[e,f], Yehuda Carmeli[g,h], David Paterson[i], Álvaro Pascual[b], Luís Martínez-Martínez[d,2], Jesús Rodríguez-Baño[b,2,*], Julián Torre-Cisneros[a,2,**], on behalf of the REIPI/ESGBIS/INCREMENT Group[3]

[a] *Instituto Maimonides de Investigación Biomédica de Córdoba (IMIBIC), Infectious Diseases Unit, Hospital Universitario Reina Sofía, Universidad de Córdoba, Cordoba, Spain*
[b] *Infectious Diseases, Clinical Microbiology and Preventive Medicine Unit, Hospital Universitario Virgen Macarena and Virgen del Rocío–IBiS, and Department of Medicine, Universidad de Sevilla, Seville, Spain*
[c] *Intensive Care Unit, Hospital Universitario Reina Sofía, Cordoba, Spain*
[d] *Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Unit of Microbiology, Hospital Universitario Reina Sofía, Universidad de Córdoba, Cordoba, Spain*
[e] *Research Service, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, OH, USA*
[f] *Departments of Medicine, Pharmacology, Biochemistry, Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, OH, USA*
[g] *Sackler Faculty of Medicine, Tel Aviv University, Israel*
[h] *National Center for Infection Control, Israel Ministry of Health, Tel Aviv, Israel*
[i] *University of Queensland Centre for Clinical Research, The University of Queensland, Herston, Brisbane, QLD, Australia*

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ABSTRACT

External validation of the INCREMENT-CPE risk score (ICS) for 30-day all-cause mortality is needed. There is also scarce information about whether colistin resistance influences the prognosis of carbapenem-resistant *Klebsiella pneumoniae* (CRKp) bacteraemia. In this study, the ability of ICS to predict all-cause mortality in the KAPECOR cohort was calculated using the area under the receiver operating characteristic (AUROC) curve. The association of colistin resistance with mortality was studied. The ICS showed an AUROC curve of 0.77 (95% CI 0.68–0.86). A cut-off of 8 points showed 96.8% sensitivity and 50.7% specificity. Mortality of low-risk patients was not different in those treated with monotherapy versus combination therapy. However, mortality of high-risk patients treated with combination therapy (37.8%) was significantly lower than in those treated with monotherapy (68.4%) ($P = 0.008$). To study the prognostic significance of colistin resistance, 83 selected cases of bacteraemia due to colistin-susceptible CRKp were obtained from the INCREMENT cohort for comparison. Colistin resistance could not be shown to be associated with higher mortality in either the high-risk ICS group [adjusted odds ratio (aOR) = 1.56, 95% CI 0.69–3.33; $P = 0.29$] or in 37 ICS-matched pairs (aOR = 1.38, 95% CI 0.55–3.42; $P = 0.49$), or in a sensitivity analysis including only KPC isolates (aOR = 1.81, 95% CI 0.73–4.57; $P = 0.20$), but the precision of estimates was low. These results validate ICS for all-cause mortality and to optimise targeted therapy for CRKp bacteraemia. Colistin resistance was not clearly associated with increased mortality.

* Corresponding author. J. Rodríguez-Baño, Present address: Unidad Clínica de Enfermedades Infecciosas, Hospital Universitario Virgen Macarena, Avda Dr Fedriani 3, 41009 Sevilla, Spain.
** J. Torre-Cisneros, Present address: Unidad Clínica de Enfermedades Infecciosos, Hospital Universitario Reina Sofía, Avda. Menéndez Pidal s/n, 14004 Córdoba, Spain.
E-mail addresses: jesusrb@us.es, jesus.rodriguez.bano.sspa@juntadeandalucia.es (J. Rodríguez-Baño), julian.torre.sspa@juntadeandalucia.es (J. Torre-Cisneros).

[1] These two authors contributed equally to this work.
[2] Members of the Spanish Network for Research in Infectious Diseases (REIPI).
[3] REIPI/ESGBIS/INCREMENT investigators are listed in the Acknowledgements section.

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1. Introduction

Carbapenem-resistant *Klebsiella pneumoniae* (CRKp) infections are associated with high mortality [1–8]. To improve the prognosis, optimising both empirical and targeted treatment is essential. The INCREMENT-CPE score (ICS), developed from a multinational cohort study [9], allows a mortality risk categorisation that might be useful for treatment decisions because it may help to select high-risk patients who would benefit from combination therapy, as shown both for the empirical treatment of CRKp colonised patients [10] and for targeted therapy [9]. However, the ability of the ICS to predict mortality or to aid in decision-making about combination targeted treatment has not been validated in an external cohort. The KAPECOR cohort includes cases of bacteraemia caused by colistin-resistant, KPC-producing CRKp conducted in the course of an outbreak in a single centre. In a previous analysis of this cohort, in which the efficacy of colistin-free regimens was shown, the only variable used to stratify the risk of mortality was the severity of systemic response (septic shock) [11].

Available options for the treatment of CRKp are usually very limited, particularly if the minimum inhibitory concentration (MIC) of carbapenems is very high. Polymyxins have been a cornerstone in the treatment of these infections [12] and, despite the recent availability of new drugs such as ceftazidime/avibactam, will still be frequently required to avoid the overuse of these newer drugs. However, resistance to colistin is increasing and it is important to evaluate the impact of colistin resistance in patient outcomes.

Therefore, the objectives of this study were to externally validate the prognostic ability of the ICS in the KAPECOR cohort and to study whether colistin resistance is related to a worse prognosis after controlling for other variables associated with the risk of all-cause mortality.

2. Materials and methods

2.1. Study design

The prognostic capacity of ICS was investigated in the KAPECOR cohort (see Section 2.2). To study whether colistin resistance is associated with a worse prognosis of CRKp bacteraemia, patients from this cohort were compared with patients with colistin-susceptible CRKp bacteraemia from the INCREMENT cohort (see Section 2.3). This report follows the STROBE recommendations [13] (see Supplementary Table S1).

2.2. KAPECOR cohort

The study design and the clinical and microbiological characteristics of the KAPECOR cohort have been described previously [11]. In summary, it is a retrospective cohort study of bacteraemia due to colistin-resistant, KPC-producing CRKp in which all isolates also showed high-level meropenem resistance (MIC $\geq$ 64 mg/L). All included patients were treated with in vitro-active regimens that were initiated in the first 5 days after extraction of the index blood culture. Patients with polymicrobial bacteraemia, those with an intra-abdominal source of infection (which are usually polymicrobial) and patients who survived <48 h after initiating active antibiotic treatment were excluded.

The KAPECOR project was approved by the Ethics Committee of the Hospital Universitario Reina Sofía (Cordoba, Spain) [code 2848], which waived the need to seek written informed consent owing to the observational nature of the study, and by the Spanish Agency for Medicines and Health Products (AEMPS) [code FIC-KPC-2015-01].

Klebsiella pneumoniae index isolates in this outbreak were previously characterised as belonging to the sequence type 512 (ST512) clone by the reference laboratory of Hospital Universitario Virgen Macarena (Seville, Spain) [11,14]. Resistance to colistin, as defined using commercial panels, was confirmed by broth microdilution following the recommendations of the Clinical and Laboratory Standards Institute–European Committee on Antimicrobial Susceptibility Testing (CLSI-EUCAST) Joint Polymyxin Breakpoints Working Group [15] in 22 of 24 available isolates (colistin MICs were 2 and 1 mg/L and 0.25 and 1 mg/L in duplicate assays for the other 2 isolates). Selected strains yielded negative screening for *mcr-1* [16] and *mcr-2* [17] genes by PCR using specific primers.

2.3. INCREMENT cohort

The characteristics of the INCREMENT cohort (ClinicalTrials.gov ID: NCT01764490) have also been published previously [9]. This is an international retrospective cohort including consecutive patients with bacteraemia caused by carbapenemase-producing Enterobacteriaceae (CPE) between 1 January 2004 and 31 December 2013. The study was approved by the Ethics Committee of the Hospital Universitario Virgen Macarena [code 1921], which waived the need to seek written informed consent owing to the observational nature of the study, and by the AEMPS [code JRB-ANT-2012-01]. To investigate the prognostic significance of colistin resistance, patients with colistin-susceptible CRKp bacteraemia treated with this drug were selected for comparison. As in the KAPECOR cohort, only patients with a vascular, urinary or pulmonary source of bacteraemia and those who started active treatment in the first 5 days after the index blood culture were selected. Patients who survived <48 h after initiating active antibiotic treatment were excluded.

2.4. Variables and definitions

The main outcome variable was all-cause mortality at 30 days after the index blood culture. The variables collected in both cohorts and their definition have been described previously [11,18]. Explanatory variables for validation of the ICS were studied on the day the blood culture was taken. The variables included in the ICS were: severe sepsis or septic shock at presentation (5 points); Pitt bacteraemia score $\geq$6 (4 points); Charlson comorbidity index $\geq$2 (3 points); source of bloodstream infection other than urinary or biliary tract (3 points); and inappropriate early targeted therapy (2 points) [18]. Inappropriate early targeted therapy was not considered here as this was an exclusion criteria (see above). Therefore, the maximum ICS was 15 points. Patients with $\geq$8 and <8 points in the ICS were considered to be at high and low risk of mortality, respectively [18].

Treatment initiated after receiving the susceptibility results was considered targeted therapy. A targeted antibiotic treatment regimen was considered active when it included at least one antibiotic to which the isolate was susceptible. In the case of gentamicin, intermediate susceptibility in vitro was accepted as this was sometimes the only available active drug in the KAPECOR cohort. The antibiotic regimens used in both cohorts have been described previously [9,11]. To classify patients as receiving a specific regimen, the regimen should have been initiated in the first 5 days following the index blood culture and maintained for $\geq$70% of the duration of treatment (or >48 h if the patient died before).

2.5. Statistical analysis

Results were expressed as the median and interquartile range for continuous variables and as number (percentage) for categorical variables. Crude comparison for continuous variables was performed using the Mann–Whitney *U*-test. For categorical variables, the Pearson's χ^2 test with Yates' continuity correction or Fisher's exact test were used as appropriate. The area under the receiver

Table 1
Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of the INCREMENT-CPE risk score for 30-day all-cause mortality in the KAPECOR cohort.

Score	Proportion of patients (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
≥3	100.00	100.0	0.0	31.0	–	31.0
≥4	86.00	96.8	18.8	34.9	92.9	43.0
≥5	86.00	96.8	18.8	34.9	92.9	43.0
≥6	84.00	96.8	21.7	35.7	93.8	45.0
≥7	65.00	96.8	49.3	46.2	97.1	64.0
≥8	64.00	96.8	50.7	46.9	97.2	65.0
≥9	49.00	80.6	65.2	51.0	88.2	70.0
≥10	48.00	77.4	65.2	50.0	86.5	69.0
≥11	45.00	77.4	69.6	53.3	87.3	72.0
≥12	19.00	32.3	87.0	52.6	74.1	70.0
≥13	10.00	16.1	92.8	50.0	71.1	69.0
≥14	10.00	16.1	92.8	50.0	71.1	69.0
≥15	10.00	16.1	92.8	50.0	71.1	69.0

Table 2
All-cause mortality of the KAPECOR cohort according to mortality risk (by ICS) and type of treatment.

Mortality risk	*n*/*N* (%)			*P*-value
	Total	Monotherapy	Combination therapy	
Low-risk mortality score (ICS 0–7)	1/36 (2.8)	0/12 (0)	1/24 (4.2)	1[a]
High-risk mortality score (ICS 8–15)	30/64 (46.9)	13/19 (68.4)	17/45 (37.8)	0.008[b]

ICS, INCREMENT-CPE risk score.
[a,b] *P*-values determined using Fisher's exact test [a] or log-rank test [b]; a *P*-value of <0.05 was considered statistically significant.

operating characteristic (AUROC) curve with the 95% confidence interval (CI) was used to quantify the discriminative capacity of the ICS to predict all-cause mortality in the KAPECOR cohort. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated in the KAPECOR cohort for different cut-off points of the ICS.

For evaluation of the prognostic significance of colistin resistance, multivariate analysis using Cox regression was attempted, but the condition of proportional risks was not met and logistic regression was therefore used. The year in which bacteraemia occurred was also considered. Variables with a *P*-value of <0.05 were considered significant. Possible interactions between variables were studied. Variables with a *P*-value of <0.1 in the univariate analyses were included in the models and were considered to be confounders if the percentage change in the coefficients was greater than 20%. The Hosmer–Lemeshow statistic was used to assess the goodness-of-fit of the model. This analysis was complemented by a conditional logistic regression analysis of a subgroup of pairs of colistin-susceptible and colistin-resistant patients matched by ICS and by use of monotherapy or combination targeted therapy, as well as by a sensitivity analysis that was performed to investigate the effect of colistin resistance in the subgroup of KPC isolates. Survival curves were obtained using the Kaplan–Meier method and were compared using the log-rank test. Analyses were performed using R v.3.0.1 and IBM SPSS Statistics v.20.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Validation of the ICS in the KAPECOR cohort: risk of 30-day all-cause mortality and efficacy of combined treatment

The KAPECOR cohort included 100 patients. The ICS applied to the KAPECOR cohort showed an AUROC of 0.77 (95% CI 0.68–0.86) (Supplementary Fig. S1), suggesting that the ICS is a good predictor of all-cause mortality risk. The sensitivity, specificity, PPV, NPV and accuracy for different cut-offs are shown in Table 1. A cut-off of 8 showed a sensitivity of 96.8% and a NPV of 97.2% but moderate specificity (50.7%) and PPV (46.9%) (Table 1).

The KAPECOR cohort was stratified according to risk of mortality by applying the ICS: 36 patients (36.0%) were classified as low risk and 64 patients (64.0%) as high risk. The crude mortality of patients classified as low risk was 2.8% (1/36), whilst that of patients classified as high risk was 46.9% (30/64) ($P < 0.001$, log-rank test). The mortality of low-risk patients treated with monotherapy was not significantly different from that observed in those treated with combination therapy [0/12 vs. 1/24 (4.2%); $P = 1$]. However, the mortality of high-risk patients treated with combination therapy was significantly lower than that of those treated with monotherapy [17/45 (37.8%) vs. 13/19 (68.4%); $P = 0.008$, log-rank test] (Table 2). Fig. 1 shows the survival curves of patients in the high-risk mortality stratum as a function of having received monotherapy or combination therapy.

A total of 44 patients from the KAPECOR cohort had septic shock, of whom 43 were classified in the high-risk group. Moreover, 18 additional patients who had severe sepsis were also classified in the high-risk group when the ICS was applied. Of these 18 patients, 11 (61.1%) were treated with monotherapy of which 8 died, and 7 patients (38.9%) received combination therapy of which 4 died.

3.2. Prognostic significance of colistin resistance

To study the prognostic significance of colistin resistance, 83 cases of bacteraemia due to colistin-susceptible CPE were obtained from the INCREMENT cohort. A flow chart of the patients included in the analysis is provided in Fig. 2. The baseline characteristics of the patients from both cohorts stratified according to the risk of 30-day all-cause mortality (by ICS) are shown in Supplementary Tables S2 and S3. Significant differences were observed in some variables potentially related to mortality risk. Patients in the KAPECOR cohort had a higher percentage of septic shock and a higher Charlson comorbidity index. Combination targeted therapy was also more frequent. Pneumonia and vascular sources of infection were significantly different between both cohorts. A stratified

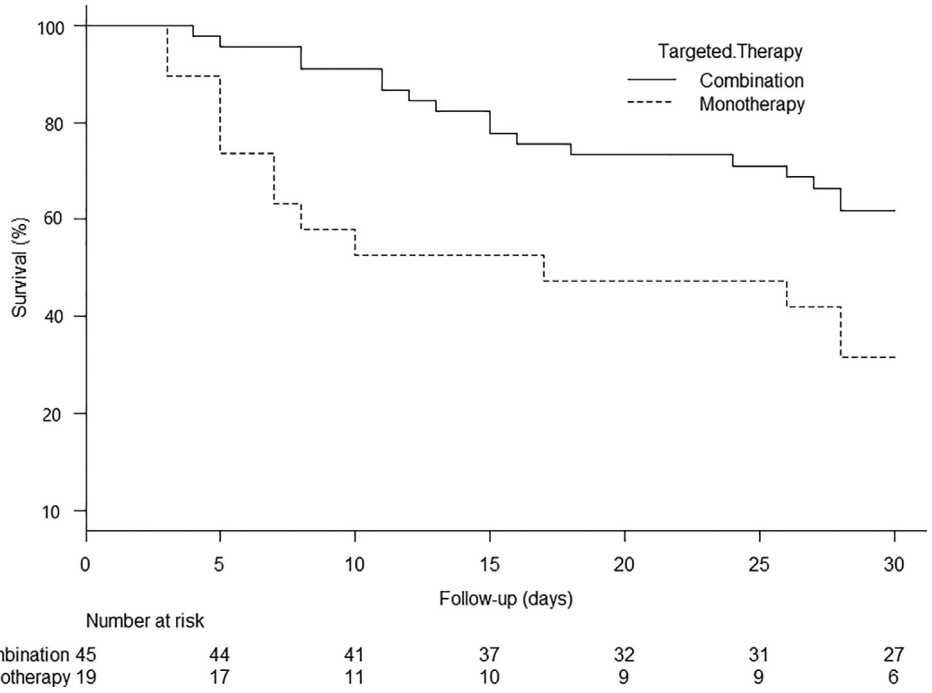

Fig. 1. Survival curves of KAPECOR patients with high risk of mortality (ICS 8–15 points) represented according to targeted treatment (monotherapy versus combination therapy). ICS, INCREMENT-CPE risk score.

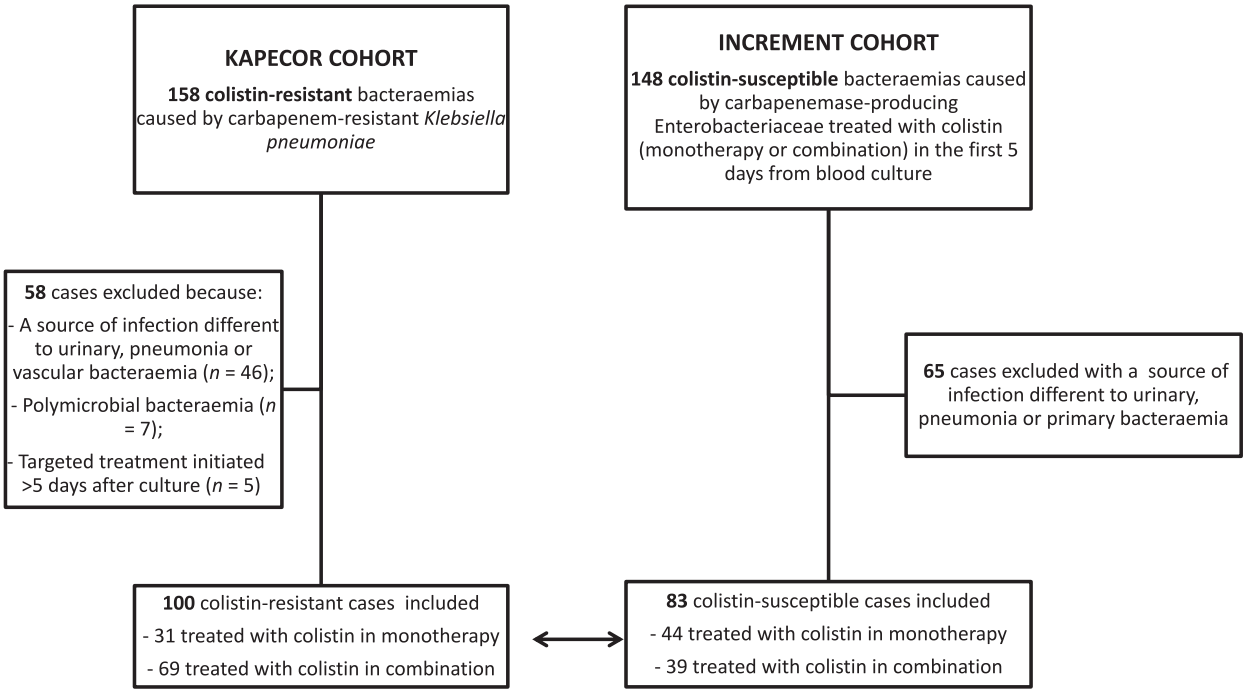

Fig. 2. Flow chart of patients from the KAPECOR and INCREMENT cohorts included in the study.

comparison of both cohorts according to ICS mortality risk is also shown in Supplementary Table S3. The treatment regimens used in the KAPECOR cohort have been described previously [11].

In the KAPECOR cohort, patients with colistin-resistant CRKp bacteraemia classified as low risk according to ICS and treated with monotherapy had a crude mortality score of 0 at 30 days (Table 2). Therefore, the prognostic significance of colistin resistance was only analysed in the subgroup of high-risk patients (109 patients) from both cohorts: 45 colistin-susceptible cases were treated with colistin in monotherapy (23 patients) or combination therapy (22 patients), and 64 colistin-resistant cases were treated without colistin in monotherapy (19 patients) or combination therapy (45 patients). Table 3 shows the univariate and multivariate analyses of 30-day all-cause mortality. In the logistic regression analysis, only combination targeted therapy was significantly protective for mortality [adjusted odds ratio (aOR) = 0.34, 95% CI 0.14–0.77; $P = 0.01$]; resistance to colistin could not be shown to be associated with higher mortality but the estimation was not precise as the 95% CI was wide (aOR = 1.56, 95% CI 0.69–3.33; $P = 0.29$). A sensitivity analysis performed to compare only KPC isolates confirmed that only combination targeted therapy was protective for mortality (aOR = 0.33, 95% CI 0.14–0.78;

Table 3
Univariate and multivariate analyses of 30-day all-cause mortality in patients with carbapenemase-producing *Klebsiella pneumoniae* bacteraemia and high mortality risk (ICS 8–15 points).

Variable	Logistic regression (109 patients)							Conditional regression (37 matched pairs)			
	Alive (60)	Dead (49)	*P*-value	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
				OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age [median (IQR)][b]	59 (47.4–71.0)	67 (52.0–73)	0.13	1.02 (0.99–1.04)	0.14			1.01 (0.97–1.04)	0.64		
Male [*n* (%)][a]	38 (63.3)	27 (55.1)	0.38	0.68 (0.31–1.44)	0.31			0.29 (0.06–1.38)	0.12		
Study period 2004–2011 [*n* (%)][a]	20 (33.3)	17 (34.7)	0.88	1.06 (0.48–2.35)	0.88			1.33 (0.46–3.84)	0.59		
KPC [*n* (%)][a]	54 (90.0)	42 (85.7)	0.49	0.65 (0.21–1.97)	0.45			0.75 (0.17–3.35)	0.71		
Colistin resistance [*n* (%)][a]	34 (56.7)	30 (61.2)	0.63	1.21 0.56–2.62)	0.63	1.56 (0.69–3.33)	0.29	1.38 (0.55–3.42)	0.49	1.38 (0.55–3.42)	0.49
Appropriate empirical therapy [*n* (%)][a]	38 (63.3)	26 (53.1)	0.38	0.71 (0.34–1.51)	0.38			0.57 (0.22–1.46)	0.25		
Delay in first active therapy from blood culture (per day) [median (IQR)][b]	1 (0–3.4)	2 (1–3.5)	0.77	1.14 (0.91–1.44)	0.25			0.97 (0.58–1.6)	0.90		
Targeted therapy [*n* (%)][a]			0.01					N/A			
Monotherapy	17 (28.3)	25 (51.0)		Ref.		Ref.					
Combination therapy	43 (71.7)	24 (49.0)		0.38 (0.17–0.83)	0.02	0.34 (0.14–0.77)	0.01				

ICS, INCREMENT-CPE score; OR, odds ratio; CI, confidence interval; IQR, interquartile range; KPC, *Klebsiella pneumoniae* carbapenemase; N/A, not applicable.
[a,b] *P*-values were determined using Pearson's χ^2 test [a] or Mann–Whitney *U*-test [b]; a *P*-value of <0.05 was considered statistically significant.

$P = 0.01$), and again resistance to colistin was not clearly associated with higher mortality (aOR = 1.81, 95% CI 0.73–4.57; $P = 0.20$). Subsequently, 37 pairs of cases matched by ICS and monotherapy or combination therapy were selected. Mortality was 14/37 (37.8%) among patients with colistin-susceptible isolates and 17/37 (45.9%) for those with colistin-resistant isolates. The estimate for the impact of colistin resistance was similar to that found in previous analyses by conditional regression (aOR = 1.38, 95% CI 0.55–3.42; $P = 0.49$) (Table 3). Supplementary Fig. S2 shows the survival curves of patients in the high-risk stratum as a function of colistin resistance in the global cohort (Supplementary Fig. S2A) and in the selected pairs (Supplementary Fig. S2B). Supplementary Fig. S3 shows the survival curves of high-risk patients treated with monotherapy and combination therapy based on colistin resistance.

4. Discussion

The results of this study support the potential utility of the ICS as a predictor of mortality in a cohort of patients with colistin-resistant CRKp bacteraemia and high-level meropenem resistance. The ICS was found to be highly predictive of mortality. When applied to the KAPECOR cohort, the predictive capacity of ICS (AUROC = 0.77) was very similar to that of the INCREMENT cohort for 30-day mortality (AUROC = 0.78 in the derivation cohort and 0.76 in the validation cohort) [18].

Because only patients with an ICS $\geq$ 8 in the INCREMENT cohort benefited from combination treatment [18], the ICS might be used in clinical practice to decide whether combination therapy is required. However, an external validation for such use was needed. The sensitivity of ICS in the INCREMENT validation cohort for mortality was 83.6% and was even higher in the KAPECOR cohort (96.8%). However, the specificity of ICS in the KAPECOR cohort was lower than that observed in the INCREMENT cohort (50.7% vs. 60.6%) [18]. This suggests that combination treatment might need to be considered for some low-risk patients; nevertheless, it would allow avoiding combination therapy for a significant subset of patients. It is striking that the mortality of KAPECOR patients with an ICS $<$ 8 treated with monotherapy was zero. It is also obvious that patients classified as high risk by ICS (ICS $\geq$ 8) benefited from combination therapy, as mortality was significantly reduced (68.4% to 37.8%; $P = 0.008$, log-rank test).

A previous analysis of the KAPECOR cohort showed that combination therapy only reduced mortality in patients with septic shock [11]. This conclusion was based on the observation of a statistical interaction between both variables, without applying the ICS. With that information, combined treatment would be indicated in 48 patients of this cohort [11]. Application of the ICS showed that 64 patients of the KAPECOR cohort had an ICS $\geq$ 8, which implies that 18 additional high-risk patients would receive combination therapy. In fact, 72.7% (8/11) of these additional high-risk patients who received monotherapy died. Therefore, our results suggest that the ICS provides useful additional information rather than simply considering septic shock. Furthermore, we recently showed that the ICS is useful to decide between monotherapy and combination therapy for empirical treatment in patients colonised with CRKp who developed an infection with a high probability of being caused by this bacteria according to the Giannella risk score [10]. A recent randomised trial found that colistin plus meropenem was not associated with lower mortality than monotherapy with colistin in infections caused by carbapenem-resistant Gram-negative bacteria [19]. However, most patients in that study had infections with *Acinetobacter baumannii* and therefore this might not apply to CRKp. Moreover, patients in the KAPECOR cohort were infected with colistin-resistant isolates.

In recent years, colistin has become a cornerstone for the treatment of CRKp infections [9,20]. The occurrence of outbreaks caused by colistin-resistant CRKp is of concern [14,21–24], mainly because some reports have associated colistin resistance with higher mortality [25,26]. The KAPECOR cohort, which includes colistin-resistant CRKp strains with high-level meropenem resistance, provided an opportunity to investigate the prognostic significance of colistin resistance in a setting of targeted therapy free of colistin and carbapenems. To achieve this objective, these patients were compared with those from the INCREMENT cohort with colistin-susceptible isolates treated with colistin and who met the same inclusion criteria as those in the KAPECOR cohort. It should be noted that patients from both cohorts showed some differences (Supplementary Table S3). The KAPECOR cohort included more severe cases with a higher risk of mortality. The proportion of patients with an ICS $\geq$ 8 was 9 percentage points higher than that of the INCREMENT validation cohort (55.2% vs. 64.0%) [11,18]. However, as this was a single-centre study conducted in the course of an outbreak and subsequent endemicity, patients could be identified early on. This translated into a very low mortality in the low-risk

patients and in the frequently correct therapeutic management of high-risk patients, both from an empirical and targeted viewpoint (combination therapy). We could not demonstrate that resistance to colistin was associated with higher mortality when the bacteraemia was monomicrobial and from a vascular, pulmonary or urinary source. In this scenario, the variable associated with mortality in the high-risk group (ICS 8–15) is type of treatment (monotherapy or combination therapy) and not resistance to colistin (Table 3; Supplementary Figs S2 and S3). Specific studies are necessary to determine whether colistin-resistant isolates are associated with lower virulence.

The development of new and more active drugs (ceftazidime/avibactam, cefiderocol, imipenem/relebactam, meropenem/vaborbactam, plazomicin and others) [27–32] will provide new, active therapeutic options for colistin-resistant, carbapenem-resistant strains. In addition, it will be necessary to study whether these drugs are even effective in monotherapy for patients with an ICS $\geq$ 8. In the future, alternative approaches to antibiotic therapy for combatting CRKp would be needed [33].

This study has the typical limitations of retrospective observational studies. The data were obtained from two cohorts with very different characteristics. Besides, the INCREMENT cohort includes Enterobacteriaceae producing KPC, OXA and some VIM carbapenemases, whilst the KAPECOR cohort only includes cases of *K. pneumoniae* producing KPC carbapenemase. Therefore, the possibility of selection bias cannot be eliminated. The number of cases is limited by the size of each cohort. To minimise the effect of these limitations, advanced statistical methods were used in the analysis, such as comparison of both groups matched by variables that have been significantly associated with mortality in these infections, as by ICS (which includes source of infection, co-morbidity and severity of infection), or by use of monotherapy or combination targeted therapy, as well as controlling the study period, or conducting a sensitivity analysis including only KPC isolates. The ICS has been validated in an external cohort with different characteristics which indicated that the ICS is a valuable tool in different clinical scenarios.

In conclusion, this study provides an external validation of the ICS and indicates that colistin resistance of CRKp strains might not worsen the prognosis if targeted treatment without colistin is early and adequate.

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The INCREMENT project investigators include: E. Salamanca and M. de Cueto (Hospital Universitario Virgen Macarena–IBis, Seville, Spain); P.-R. Hsueh (National Taiwan University Hospital, Taiwan); P. Viale, M. Bartoletti and M. Giannella (Teaching Hospital Policlinico S. Orsola Malpighi, Bologna, Italy); J.R. Paño-Pardo, M. Mora-Rillo and C. Navarro-San Francisco (Hospital La Paz–IDIPAZ, Madrid, Spain); M. Venditti, M. Falcone and A. Russo (Policlinico Umberto I, Rome, Italy); M. Tumbarello, E.M. Trecarichi and A.R. Losito (Catholic University of the Sacred Heart, Rome, Italy); G. Daikos and A. Skiada (National and Kapodistrian University of Athens, Laikon General Hospital, Athens, Greece); R. Cantón, V. Pintado and P. Ruiz (Hospital Ramón y Cajal, Madrid, Spain); Y. Doi (University of Pittsburgh, Pittsburgh, PA, USA); F.F. Tuon (Hospital da Universida de Federal do Paraná, Curitiba, Brazil); I. Karaiskos and H. Giamarellou (Hygeia General Hospital, Athens, Greece); M.J. Schwaber (Sackler Faculty of Medicine, Tel Aviv University and National Center for Infection Control, Israel Ministry of Health, Tel Aviv, Israel); Ö.K. Azap (Başkent University Faculty of Medicine, Ankara, Turkey); M. Souli, A. Antoniadou and G. Poulakou (University General Hospital Attikon, Chaidiri, Greece); E. Roilides and E. Iosifidis (Hippokration Hospital, Thessaloniki, Greece); S. Pournaras, A. Tsakris and O. Zarkotou (Medical School, National and Kapodistrian University of Athens, Athens, Greece); M. Akova, Ö. Helvaci and A.O. Sahin (Hacettepe University, Ankara, Turkey); F. Pérez (Research Service, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, OH, USA); J. Bermejo and V. Rucci (Hospital Español, Rosario, Argentina); A. Oliver, E. Ruiz de Gopegui and C. I. Marinescu (Hospital Universitario Son Espases, Palma de Mallorca, Spain); C. de la Calle, J.A. Martínez, L. Morata and A. Soriano (Hospital Clinic, Barcelona, Spain); W. Lowman (Wits Donald Gordon Medical Centre, Johannesburg, South Africa); B. Almirante, N. Larrosa and M. Puig-Asensio (Hospital Universitario Vall d'Hebrón, Barcelona, Spain); E. García-Vázquez, A. Hernández and J. Gómez (Hospital Universitario Virgen de la Arrixaca, Murcia, Spain); G. Bou (Complejo Hospitalario Universitario A Coruña, A Coruña, Spain); N. Prim, F. Navarro and B. Mirelis (Hospital de la Santa Creu i Sant Pau, Barcelona, Spain); J. Origüen, R. San Juan and M. Fernández-Ruiz (Hospital 12 de Octubre, Madrid, Spain); J.M. Cisneros, J. Molina and V. González (Hospital Universitarios Virgen del Rocío-IBis, Seville, Spain); M.C. Fariñas, M.E. Cano and M. Gozalo (Hospital Universitario Marqués de Valdecilla–IDIVAL, Santander, Spain); C. Peña, S. Gómez-Zorrilla and F. Tubau (Hospital Bellvitge, Barcelona, Spain); J. Pitout and D. Virmani (University of Calgary, Calgary, Canada); C. Natera and E. Marfil (Reina Sofía University Hospital–IMIBIC, University of Cordoba, Cordoba, Spain); E. Tacconelli and F. Riemenschneider (Universitätsklinikum Tübingen, Tübingen, Germany); E. Calbo, C. Badia and M. Xercavins (Hospital Universitario Mútua de Terrassa, Terrassa, Spain); and O. Gasch, D. Fontanals and E. Jové (Hospital Parc Taulí, Sabadell, Spain).

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Competing interests

JR-B has served as a scientific advisor for research projects for AstraZeneca, Pfizer and InfectoPharm, and has been a speaker in unrestricted accredited educational activities funded by Merck. All other authors declare no competing interests.

Ethical approval

The KAPECOR project was approved by the Ethics Committee of the Hospital Universitario Reina Sofía (Cordoba, Spain) [code 2848], which waived the need to seek written informed consent owing to the observational nature of the study, and by the Spanish Agency for Medicines and Health Products (AEMPS) [code FIC-KPC-2015-01]. The INCREMENT study was approved by the Ethics Committee of the Hospital Universitario Virgen Macarena [code 1921], which waived the need to seek written informed consent owing to the observational nature of the study, and by the AEMPS [code JRB-ANT-2012-01].

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.07.017.

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