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ORIGINAL ARTICLE

Colistin resistance increases 28-day mortality in bloodstream infections due to carbapenem-resistant *Klebsiella pneumoniae*

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Abstract

Mortality due to *K. pneumoniae* bacteremia is on rise, particularly in regions with high rates of carbapenem and colistin resistance. We aimed to define risk factors for colistin resistance and its impact on mortality. Patients diagnosed with "carbapenem-resistant *K. pneumoniae* (CRKp)" bacteremia between 2014 and 2018 were divided into two groups as "colistin susceptible (ColS)" and "colistin resistant (ColR)" based on broth microdilution method. Retrospective case-control study was conducted to compare characteristics and outcomes. Multiple logistic regression model was used to define independent risk factors for acquired colistin resistance and Cox proportional hazard model for 28-day mortality. A total of 82 patients (39 ColS and 43 ColR) were included. Mean age was 61.5 years, and 50 (61%) were male. Colistin resistance was significantly increased with duration of hospital stay ($p = 0.007$) and prior colistin use ($p = 0.007$). Overall, the 28-day mortality rate was 66%. Age ($p = 0.014$) and colistin resistance significantly increased 28-day ($p = 0.009$) mortality. Microbiological response to treatment within 7 days favors survival. PFGE analysis revealed an outbreak with *K. pneumoniae* ST78 and ST45 clones. Patients treated with combined antimicrobials had significantly lower 28-day mortality ($p = 0.045$) in comparison to monotherapy. However, types of combinations did not show significant superiority on each other. Colistin resistance increases 28-day mortality in CRKp bacteremia. Although combined regimens are more effective than monotherapy, existing antibacterial combinations have no apparent superiority to each other. New treatment options are pivotal.

Keywords Colistin resistance · Carbapenem resistance · *Klebsiella pneumoniae* · Bloodstream infection · Mortality

Introduction

The increasing incidence of colistin-resistant (ColR) *Klebsiella pneumoniae* (Kp) strains as bloodstream pathogens is a matter of concern due to unfavorable outcomes and lack of treatment options. With the rise in consumption of colistin, cases of colistin-resistant *Klebsiella pneumonia* strains are reported globally [1]. Since 2013 colistin resistance rate has increased up to one-third of carbapenem-resistant isolates in Europe, and recent reports point out an ongoing rise [2–4]. According to the WHO-CAESAR 2017 report, ertapenem resistance has reached to 43% among *Klebsiella pneumoniae* isolates in Turkey, and recent reports draw attention to a rapid emergence of colistin resistance with high fatality rates [5, 6].

Researches indicate that colistin resistance may reach 76% among carbapenem-resistant Enterobacteriaceae (CRE) in Turkey [7].

In our center, one of the largest tertiary hospitals in Istanbul, carbapenem resistance has emerged in *K. pneumoniae* in 2011, and a series of 36 cases with carbapenem-resistant (CR) *Klebsiella pneumoniae* bacteremia has been published in 2014 [8]. The 30-day mortality rate was 50%, very similar to some other cohorts in Europe [9, 10]. Colistin-resistant *K. pneumoniae* with high-level (MIC > 64 mcg/mL) carbapenem resistance as bloodstream pathogens were first introduced at the beginning of 2014 and caused the highest recorded fatalities (77% for the first 35 cases) in the following years. An average of 115 cases with bloodstream infections due to *K. pneumoniae* have been identified annually between 2014 and 2018 of whom the rates of ESBL,

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CR, and ColR were of 75.62%, 60.8%, and 23.46%, consecutively.

To date, the best treatment regimen for bloodstream infections due to colistin-resistant *K. pneumoniae* with high-level carbapenem resistance is unknown. Here, we analyzed the 5-year data from a tertiary center in a OXA-48 dominant region and aimed to evaluate the epidemiological characteristics of risk factors for blood stream infections due to ColR-Kp, determine the molecular epidemiology of these strains, examine the variables associated with poor outcomes, and unveil the relation between colistin resistance and mortality. A retrospective case-control analysis was conducted to compare the outcomes of patients with bloodstream infections due to ColR-CRKp versus colistin-susceptible (ColS) CRKp.

Methods

Setting

Cerrahpaşa Medical School is a 1100 bed tertiary-referral teaching hospital in the center of Istanbul, the most populous city of Turkey.

Approximately 910,000 patients (adult and pediatric) admit to the hospital annually. Of these, 160,000 are hospitalized for an average stay of 2.8 days. As part of infection control measures, routine surveillance for carbapenem-resistant *K. pneumoniae* is performed (via rectal screening upon admission and weekly until discharge) for patients admitting to intensive care units (ICUs), hematology, and infectious diseases wards.

Microbiology

CRKp were defined as *K. pneumoniae* isolates with nonsusceptibility to any of the following carbapenems: meropenem, imipenem, or ertapenem, as outlined by the Clinical and Laboratory Standards Institute (CLSI) *(Clinical and Laboratory Standards Institute*) and EUCAST *(European Committee on Antimicrobial Susceptibility Testing)* [11, 12]. Strains stored in glycerin-containing Brucella broth at − 20 °C were tested with cation-adjusted Mueller Hinton-based broth microdilution method using colistin sulfate and grouped as ColS or ColR in accordance of the microdilution sensitivity results.

Broth microdilution methodology After being purified, all strains were stored in glycerin-containing Brucella broth at − 20 °C until the study was performed. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 strains were used for quality control. Colistin sulfate (COS) was supplied from the manufacturers in powder form and stored in accordance with the manufacturer's recommendations. In order to prepare stock solution, it was diluted with distilled water to a final concentration of 1280 μg/mL according to the manufacturer's recommendations, and 500 μL was distributed to each Eppendorf and stored at − 20 °C. During the study procedure, the stock solutions were diluted with cation-adjusted Mueller Hinton broth (cation-adjusted Mueller Hinton broth/CAMHB) and distributed to falcon tubes as described in the CLSI as the final concentration in the plates for both antibiotics was 16–0.015 μg/mL. One tube for each concentration and each drug, 11 tubes in total were used. Drug-free CAMHB was placed in a falcon tube to be dispensed into growth control wells. Seven strains were tested in each microplate, and in the last row three wells were used as sterility control wells, and 200 μL of drug-free CAMHB were distributed to each. Medicated solutions in Falcon tubes were dispensed 100 μl into each well so that the concentration decreases from the 1st column to the 11th column. One hundred microliter of drug-free CAMHB was also dispensed to the wells in the twelfth column. The prepared microplates were stored in a freezer at − 20 °C until the testing procedure. Strains stored in Eppendorf were prepared for testing by taking two passages (subculture) for 24 h. First, a 0.5 McFarland (2.5–5 × 108 bacteria) turbidity solution was prepared as described in the CLSI standards. Later, first 1/100 and then 1/10 ratio (1–2.5 × 105 bacteria) were diluted with CAMHB, and 100 μL was distributed to all wells of the plates that were taken out of the freezer and rested, except the sterility control wells. After 24-h incubation at 37 °C, the first well without growth was evaluated as the MIC value. In vitro polymyxin resistance was defined per European Committee on Antimicrobial Susceptibility Testing guidelines as MIC of > 2 mg/L [12]. Very major and major error rates were defined as described elsewhere [13]. Pulse-field gel electrophoresis (PFGE) analysis was conducted to establish the molecular similarities of the strains. Multi-locus sequence typing (MLST) was used to identify the sequence types of the major strains related to the outbreak. Molecular analyses were performed to search for carbapenemase genes. Pan-primers were used to detect OXA-48-, KPC-, VIM-, IMP-, and NDM-type carbapenemases.

Patients

Patients were selected upon records of Infectious Diseases and Clinical Microbiology Laboratory that gives routine service for adult patients followed in ICUs and internal medicine wards. Patients with BSIs due to CRKp (meropenem MIC > 2 mg/L or ertapenem MIC > 0.5 mg/L) between January 01, 2014, and June 01, 2018, were included. A total of 109 patients had positive blood cultures for CRKp within the given time period, of whom 27 were excluded for various reasons and the remaining 82 consecutive subjects were included in the study, as shown in the consort diagram (Fig. 1).

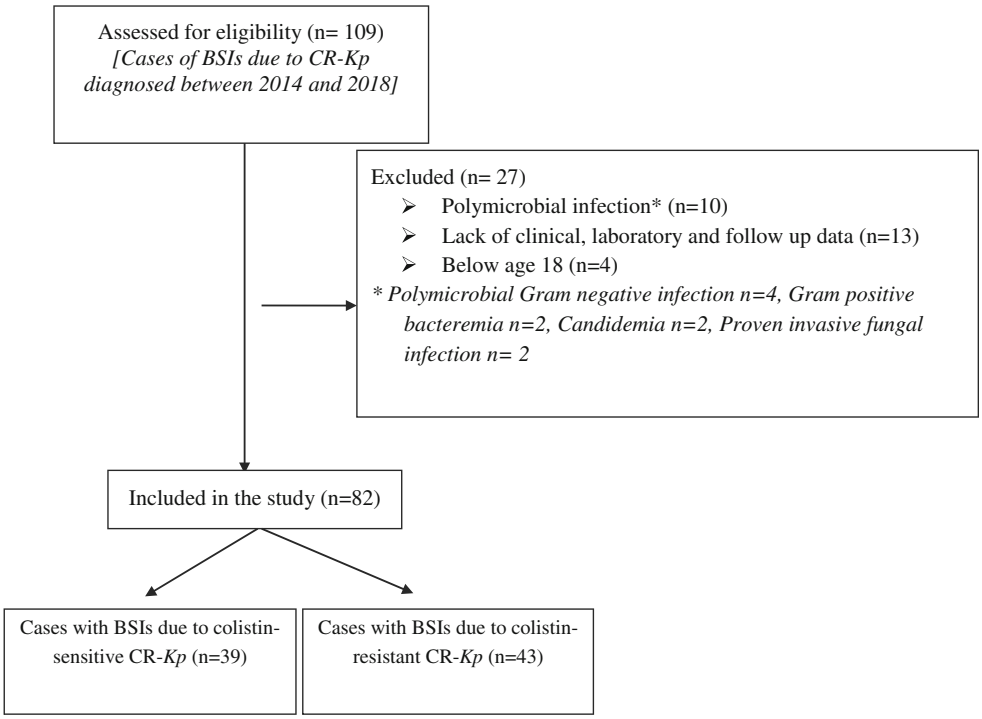

Fig. 1 Consort diagram

Statistical analysis

Eighty-two cases with CR *K. pneumoniae* bacteremia were divided into two groups as ColR and ColS. The two groups were compared in terms of demographic, epidemiological, microbiological characteristics, and treatment results. Differences between the two groups were investigated by multiple logistic regression analysis. In order to construct this model appropriately, the relationships between the variables that are likely to be effective in the emergence of colistin resistance were evaluated by appropriate univariate analyzes (chi-square test or one-way ANOVA model), and the variables with a p value of <0.30 in these analyses were included in multiple logistic regression model. The reasons for including p values between 0.05 and 0.3 were the probability of being significant in multivariate models, having a biological meaning or having a confounder effect. This method is defined as "purposeful variable selection method" and is suggested in order to identify the confounders more clearly and diminish their effects particularly when the number of variables is high and the sample size is relatively small [14]. The relationships between the risk factors that might affect 28th day survival were examined by Kaplan-Meier analysis. Variables with p value <0.30 were included in a multiple Cox regression model and re-analyzed, based on the rationale as stated above. For checking the proportional hazard assumption of the Cox proportional hazard model, we generated the time-dependent covariates by creating interactions of the predictors and a function of survival time and included in the model. The final significance level of p value of the risk factors was taken as <0.05 in both multiple logistic and Cox regression analyses. All statistical analyses were done by using IBM SPSS for Windows (Ver.22.0) [15].

Outcome measures

Twenty-eight-day survival was the main outcome measure to compare the two groups (CRKp and ColR-Kp bacteremias) in terms of antimicrobial treatment results.

Results

Results of molecular analyses

Of the 82 strains included in the case-control study, only 67 were able to be preserved and included for molecular analysis. PFGE results revealed that all 67 strains were correlated with each other (Fig. 2), according to Tennover's $\geq 85\%$ similarity criterion. Ninety percent of the strains consisted of a single major clone those divided into three pulsotypes: group I consisted of 60 strains, group II covered 2 strains, and group III comprised 5 strains. The *K. pneumoniae* isolates related to the outbreak were characterized as belonging to the sequence type (ST)78 and ST45 clones, according to the MLST analyses. Carbapenemase genes were investigated with polymerized

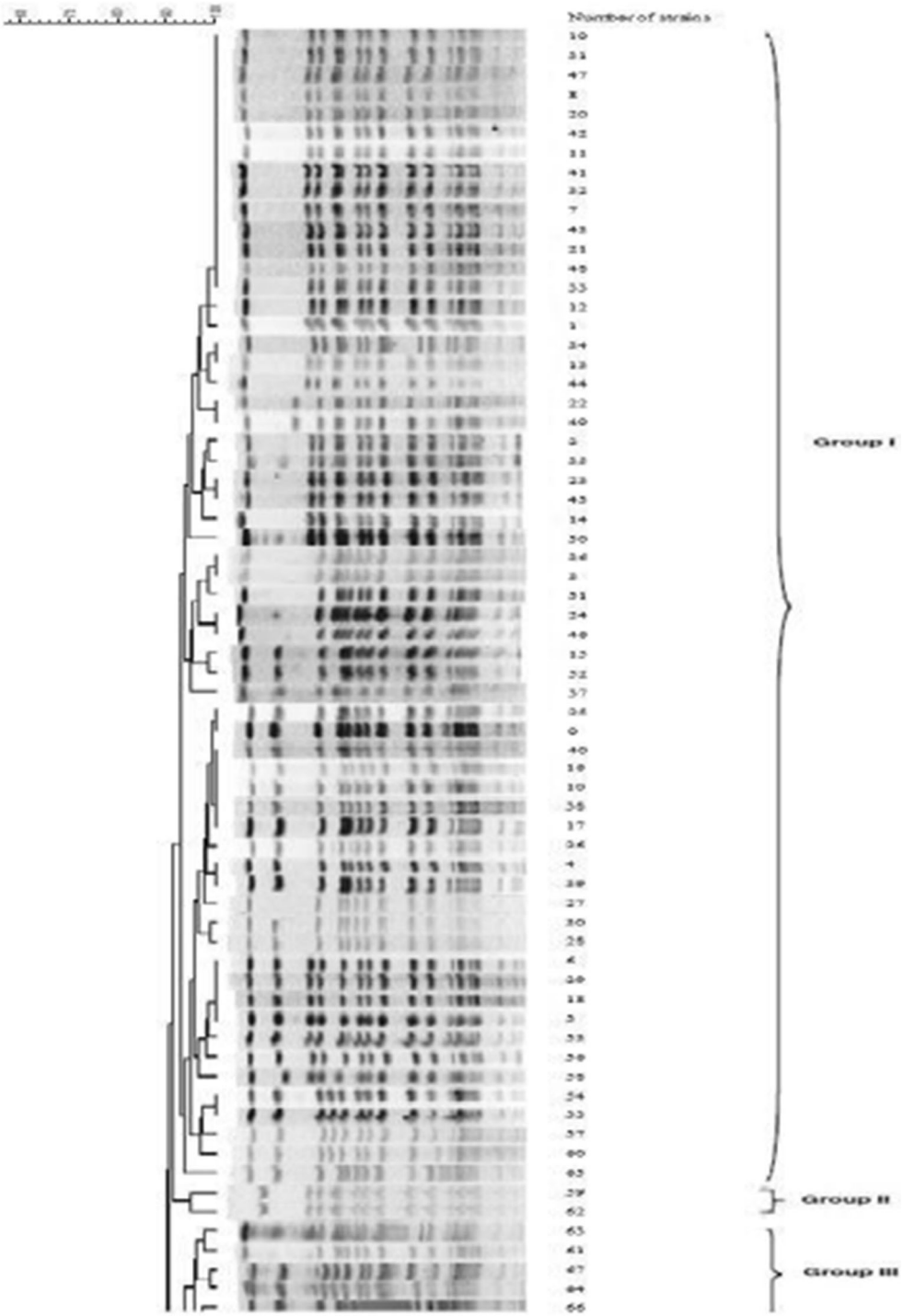

Fig. 2 PFGE dendrogram

chain reaction (PCR). OXA-48-like carbapenemase genes were detected in 40 of the total 67 strains (of which colistin resistance was negative in 15 and positive in 25). The remaining 27 strains were found negative in terms of KPC-, VIM-, IMP-, and NDM-type carbapenemases. Carbapenem resistance in these strains was attributed to over-expression of other beta-lactamases, porin loss, and efflux mechanisms, hypothetically.

Risk factors leading to colistin resistance

Of the 82 patients included in the study, 50 were male (61%), 60 (73.2%) were hospitalized in intensive care units, and 22 (27%) were hospitalized in internal medicine clinics. Colistin resistance was present in 55% of ICU patients and 45.5% of internal medicine patients. The difference was not statistically significant ($p = 0.466$). The risk factors for emergence of

colistin resistance those found as significant in simple binary logistic regression and selected for multi-model are given in Table 1. ROC analysis was performed for the multivariate logistic regression model (Fig. 3). The area under curve (AUC) was determined as 0.931 ± 0.029 ($p < 0.001$). This result demonstrates the success of the model given in Table 1 to distinguish between those with and without colistin resistance. The rate of colistin resistance significantly increased (1.033 fold, $p = 0.015$) for each day of prolonged hospital stay before bacteremia. Exposure to colistin within the last 3 months was significantly (43.1 fold, $p = 0.006$) more common in those with colistin resistance. Prior rectal colonization with CRKp ($p = 0.05$) and history of intra-abdominal surgery within last 3 months ($p = 0.014$) were significantly lower in colistin-resistant cases. Presence of OXA-48-type carbapenemase was not significant in predicting the development of colistin resistance ($p = 0.3$).

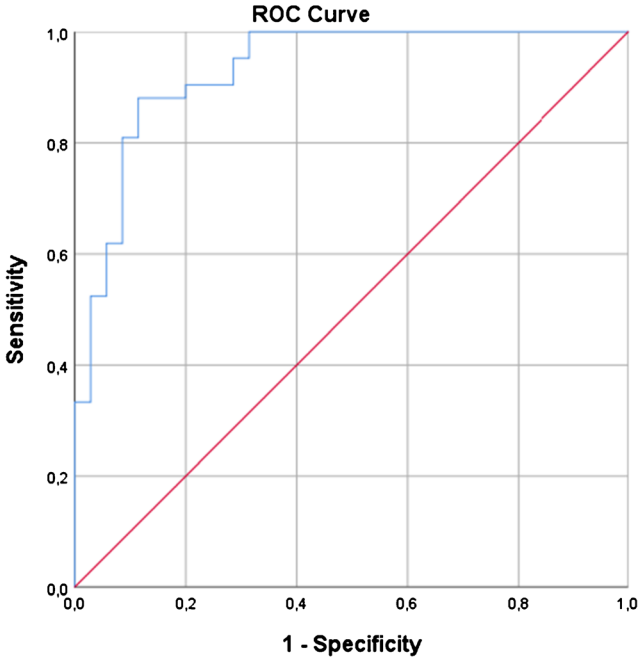

Fig. 3 ROC curve for the multivariate logistic regression model

Risk factors affecting 28-day survival

Of the 82 patients, 37 survived on day 14 and 28 survived on day 28. The risk factors affecting 28-day survival those found as significant in simple binary logistic regression and selected for multi-model are given in Table 2. We performed checks on the assumption of proportionality of hazards. Since all the time-dependent covariates were not statistically significant ($p > 0.05$), proportional hazards assumption of the model was valid. The ROC curve drawn for the Cox proportional hazard model is given in Fig. 4. AUC has been found as 0.848 ± 0.048 ($p < 0.001$) which demonstrates the acceptable success of the model given in Table 2 to distinguish between survivors and non-survivors on day 28. Every single year of aging increased the risk of mortality by 1.041

Table 1 Multivariate analysis of risk factors for colistin-resistant CR-*K. pneumoniae* bloodstream infection

Continuous type risk factors		Colistin susceptible (n =39)		Colistin resistant (n = 43)		OR (adjusted)	95% confidence interval (CI)		p
							Lowest	Highest	
Age		61.5±17.2		61.5±17.6		1.033	0.953	1.119	0.428
Charlson Comorbidity index		6.95±2.91		5.58±3.13		0.808	0.486	1.345	0.412
Duration of hospital stay preceding bacteremia (days)		38.67±36.51		70.49± 64.2		1.033	1.006	1.061	*0.015*
Duration of ICU stay preceding bacteremia (days)		20.95±30.1		20.93± 33.05		0.979	0.947	1.012	0.214
Risk factors of categorical type	Risk category	n	%	n	%	OR (adjusted)	95% CI Lowest	Highest	p
Gender*	Female	15	38.5	17	39.5	1.881	0.314	11.256	0.489
Clinical ward*	Internal medicine wards	12	30.8	10	23.3	0.116	0.007	1.847	0.127
Solid tumor*	Yes	13	33.3	8	18.6	5.420	0.210	139.627	0.308
Hematological malignancy*	Yes	9	23.1	11	25.6	0.506	0.034	7.437	0.620
CRE colonization before bacteremia*	Yes	23	65.7	21	50.0	0.148	0.019	0.999	*0.050*
Carbapenem use within the last 3 months*	Yes	28	71.8	38	88.4	6.140	0.385	98.003	0.199
Colistin use within the last 3 months *	Yes	8	20.5	27	62.8	43.101	2.970	625.601	*0.006*
Surgical operation use within the last 3 months *	Intra-abdominal surgery	19	48.7	14	32.6	0.004	0.001	0.329	*0.014*
	Other surgeries	8	20.5	7	16.03	0.165	0.006	4.195	0.275
Presence of OXA-48	Yes	15	38.5	25	58.1	2.518	0.436	14.538	0.302

*: In the comparisons, Male, Intensive care hospitalization, and None categories were accepted as reference category

Table 2 Multivariate analysis of risk factors affecting 28-day survival *(survived n = 28, mortality on day 28 n = 54)*

Risk factors	Risk/reference category*	Adjusted HR	95% CI		*p*
			Lower	Upper	
Age		1.041	1.013	1.070	*0.004*
ICU stay preceding bacteremia	Yes/no	1.730	0.602	4.971	0.309
Antibiotic use within the last 3 months	No/yes	5.407	0.478	61.190	0.173
Surgery within the last 3 months	Abdominal surgery: Yes/no	1.613	0.642	4.050	0.309
	Other surgeries Yes/no	0.984	.303	3.196	0.978
Removal of infected central venous catheter *(in case of indication)*	Removed/not removed	0.047	0.006	0.355	*0.003*
Colistin resistance	Yes/no	3.700	1.459	9.387	*0.006*
Targeted combination therapies	Double combination/monotherapy	0.311	0.097	0.995	*0.049*
	Triple combination/monotherapy	0.342	0.077	1.525	0.159
	Quadruple combination/monotherapy	0.356	0.064	1.989	0.239
Microbiological response within 7 days	No/yes	2.484	1.080	5.711	*0.032*
Presence of OXA-48-like carbapenemase	Yes/no	1.980	0.785	4.998	0.148

HR hazard ratio

times ($p = 0.004$) within 28 days. Risk of mortality within 28 days was significantly increased (adjusted hazard ratio (aHR), 0.047; 95% confidence interval (CI), 0.006–0.355; *p = 0003*) in cases where infected catheters were not removed in comparison to those removed. Empirical treatment modalities, whether monotherapy or combination, did not differ significantly in terms of effect on 28-day survival. However failure to start targeted treatment within 72 h after obtaining blood cultures significantly increased 28-day mortality ($p = 0.001$). Regarding targeted treatment modalities, a total of 76 patients were able to receive treatment and six patients died before starting targeted antibacterial therapy. Colistin was used in 36 cases, 18 (50%) in ColS and 18(50%) in ColR groups, combined with a carbapenem (double combination in 15, triple combination in 21) in all. Combination treatment was more commonly ($p = 0.009$) preferred in ColR group (60.5%), compared to ColS group (30.8%), and 28-day mortality was significantly lower in patients treated with combined antimicrobials in comparison to monotherapy ($p = 0.049$). However, types of combinations did not show significant superiority on each other. Colistin resistance was associated with a 3.7 fold increase in 28-day mortality (95% CI; 1.459–9.387, $p = 0.006$). Adjusted Kaplan-Meier survival curves censored at day 28 according to the presence of colistin resistance is shown in Fig. 5. Patients who became culture negative within 7 days of treatment had a significantly lower risk of mortality (adjusted HR = 2.484, CI *p = 0.032*) at 28 days. Duration of hospital or ICU stay before bacteremia, prior rectal colonization with CRKp, history of surgery within the last 3 months, use of carbapenem within the last 3 months, and type of infection causing bacteremia did not show significant effect on 28-day survival. Similarly, OXA-48-like carbapenemase positivity was also not significantly associated with increased risk of mortality at day 28 ($p = 0.148$).

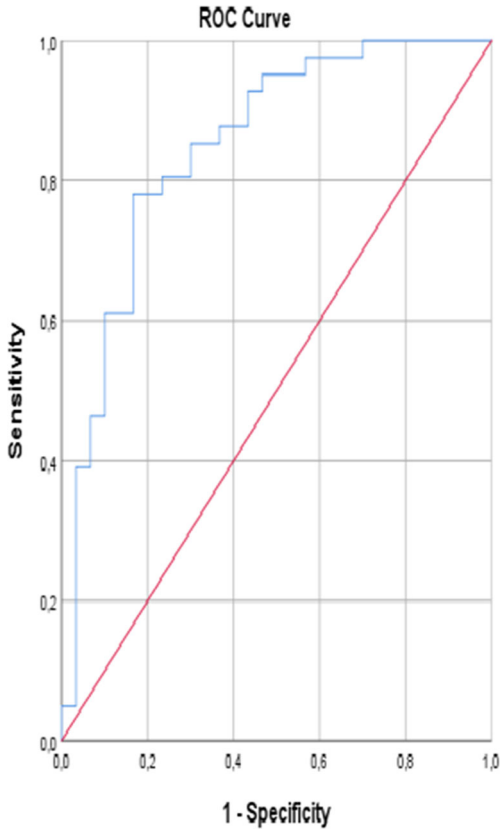

Fig. 4 ROC curve for the Cox proportional hazard model

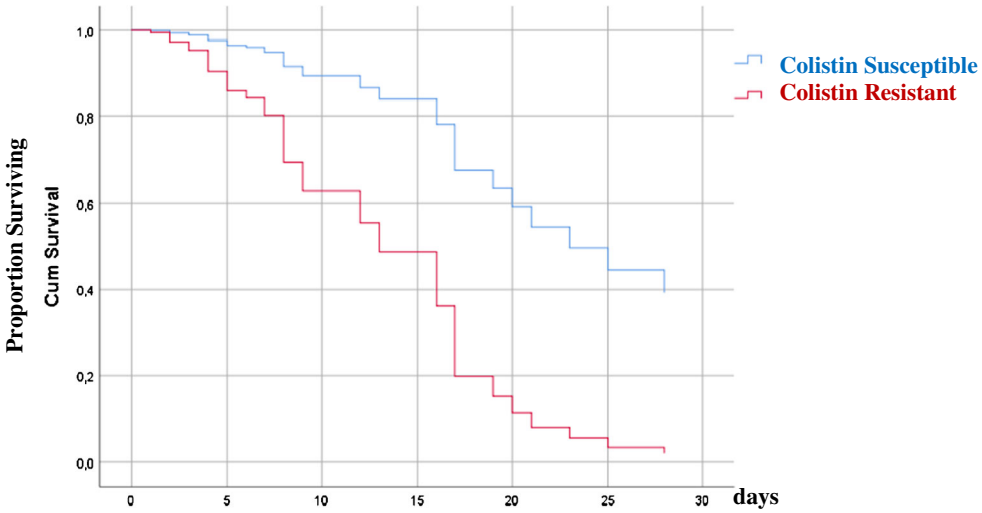

Fig. 5 Kaplan-Meier curves demonstrating 28-day survival for patients with BSIs due to colistin-susceptible and colistin-resistant CRK

Discussion

The question about the role of colistin resistance in the increasing mortality rates due to CRKp bacteremia have partly been elucidated with this study. Colistin resistance increases 28-day mortality rates in CRKp bacteremia. Overall treatment success basically depends on prompt and sufficient source control rather than preferring any of existing antibacterial combinations. Microbiological response to treatment within 7 days is strongly predictive for 28-day survival.

Risk factors predisposing colistin resistance were also evaluated in the study. Prior duration of hospital stay was found to be significantly longer, and exposure to colistin within last 3 months was determined as a major risk factor in patients with colistin resistance. Duration of hospital stay could be a confounding factor for carbapenem and colistin use. However, results of multivariate analyses showed that prior exposure to colistin is independently and strongly associated with emergence of colistin resistance. The significant temporal association between colistin use and colistin resistance has been identified in many earlier studies [16–18]. According to the study of Tansarli et al., the time interval between the effect of colistin use on the grow of subsequent colistin resistance was about 3 months. Prior exposure to colistin could explain 69% of colistin resistance; and other factors might play a role in the remaining 31%. Colistin resistance is caused by mutations of genes in lipopolysaccharide biosynthesis pathways that are most likely driven by the selection pressure exerted by colistin [19–21].

The role of previous colistin therapy as an independent risk factor for BSIs due to ColR Kp underscores the absolute need of avoiding unnecessary colistin use, suboptimal dosing, or prolonged monotherapy [19]. This requires the application of strict rules for the initiation and, perhaps more importantly, early discontinuation of colistin in clinical practice particularly in hospitals that are endemic for CR Kp infections [18].

PFGE results in our study revealed one major clone for the 90% of the strains, which highly implies the presence of cross-transmission of endemic strains between patients, particularly between the years 2014 and 2016. The major clone contains strains from three pulsotypes isolated at scattered time points, suggesting the high probability that they are resident inhabitants of the ICU biogeography. The apparent monoclonal nature of the isolates in our cohort suggests direct patient-to-patient transmission within these units, and this is likely to be the primary factor driving resistance rates. The results further indicate that current infection control measures are vulnerable and might result in outbreaks due to ColR strains. The lower incidence of prior CRE colonization among ColR cases in our cohort supports our conclusion, because in cases with a pre-defined CRE colonization, contact isolation precautions are applied more strictly to prevent cross-contamination.

In our study, the mortality rate clearly appeared to be higher in ColR CR-Kp BSIs compared to ColS cases (51% vs. 39%), consistently with some previous reports [1, 22, 23]. This can be explained by an inevitable delay in detection of true colistin resistance and by the low activity and pharmacokinetic weakness of some available treatment options [24]. Although colistin resistance was not clearly associated with increased mortality in the INCREMENT study, many studies report different results. In an outbreak investigation carried out in 2016 in Turkey in a 550-bed tertiary care hospital, all five cases of infection due to colistin-resistant *K. pneumoniae* died, with a 14-day mortality rate of 60% and a 30-day mortality rate of 100% [25, 26]. These findings were confirmed by a large prospective cohort study performed in 2015–2016 including 115 patients diagnosed with colistin-resistant *K. pneumoniae* infections [27]. The study reported an overall 30-day mortality rate of 61%. An Italian prospective study on patients with MDR *K. pneumoniae* revealed that infection due to a ColR *K. pneumoniae* strain was independently associated with higher mortality (odds ratio (OR) 4.14, 95% CI 1.17–14.74, $p < 0.02$) [22].

Because colistin, more than other drugs (tigecycline and aminoglycosides), is the backbone of therapy for CR-Kp infections, colistin resistance reduces the already poor therapeutic armamentarium for these patients. Studies to define molecular epidemiology of infections due to ColR strains and to adequately weigh the role of antimicrobial therapy against this challenge are critical [28, 29]. The results of monotherapy and combinations in empirical treatment were similar in our cohort regardless of which drugs were used. However, mortality was significantly higher in patients who did not receive targeted treatment within 72 h after bacteremia. Despite lack of infection severity scores, our cohort provides further evidence for previously defined predictors of mortality such as older age and sepsis or septic shock at the onset [30].

In some studies, pneumonia has been reported as a focus of bacteremia associated with higher fatality rates [29]. However, we found no significant difference between the foci of infection in our cohort, in terms of fatality. The foremost predictor of 28-day survival was the microbiological response to targeted therapy within the first 7 days in our study, as highlighted in results of several other studies [8, 31].

Appropriate antimicrobial treatment, including combinations of active antimicrobials and removal of catheter in catheter-related bacteremia, is associated with higher survival in several studies in compliance with our results [30]. It has been demonstrated that combined therapy is superior to monotherapy in CR and ColR-Kp bacteremia, especially in septic shock [32–34]. Combination therapy with two in vitro active agents, mostly colistin plus amikacin, showed a survival benefit compared with other regimens in critically ill patients [9].

In our study, combination treatment was more commonly ($p = 0.009$) preferred in ColR group (60.5%), compared to ColS group (30.8%), and 28-day mortality was significantly lower in patients treated with combined antimicrobials in comparison to monotherapy ($p = 0.049$). However, any combination modalities revealed no apparent superiority to each other, and colistin resistance had a more decisive effect on clinical outcome. In our cohort, stratification could not be performed in terms of treatment success because clinical severity scores were not available for most cases.

There are studies in the literature indicating higher survival rates with carbapenem-containing combinations even in cases with carbapenem resistance [32, 35]. Although it is not applicable for ColR and high-level CR (meropenem MIC > 64) strains, carbapenems were mostly involved in the treatment combinations for such cases in our center. Ceftazidime-avibactam was not yet available in our country within the study period, and fosfomycin IV had very recently been introduced.

This study has several limitations. It is a single-center study so any extrapolation warrants caution. We could not include all 82 strains for the molecular study due to insufficiencies in strain collection and preservation conditions. Due to lack of data, the clinical severity scores of the cases were not available for comparative analyses. Previous antibiotic exposure was also not sufficiently documented to establish any relationship between use of antibiotics other than colistin and growing colistin resistance. The study was not randomized, and despite attempts to control for confounders by using multivariate analysis, residual confounding might have occurred. Lack of standard broth microdilution results at the time of treatment was another major limitation which caused false-susceptible results (very major error) in six strains. However, colistin was the most common backbone antimicrobial in both ColS and ColR groups.

The study was performed in the context of an outbreak. Finally, the sample size, which was constrained by the available cases, is a further limitation of the analysis.

Conclusion

This study emphasizes the emergence of resistance to colistin, the last weapon remaining in the antibiotics arsenal for carbapenem-resistant *Klebsiella pneumoniae* infections in Turkey. Colistin resistance increases in-hospital mortality rates in CRKp bacteremia. Existing antibacterial combinations have no apparent superiority to each other, regardless of molecular type of carbapenemases. Microbiological response to treatment within first 7 days of treatment favors survival. Horizontal transmission owing to insufficient infection control measures is among major drivers of increasing resistance rates, which brings us one step closer to a post-antibiotic era crisis. Strict prevention of cross-transmission besides introducing new treatment options is pivotal to overcome the daunting consequences of colistin resistance.

Acknowledgments We commemorate Nur Hondur with mercy and respect and express our sincere appreciations to Hatice Yaşar Arsu and Zeynep Alişan for their dedication to ensure that every work in the laboratory is carried out to the highest quality. Heartfelt thanks to Songül Gedik Koç for her extraordinary efforts to retrieve patient data. We are grateful to Prof. Recep Öztürk for establishing Infectious Diseases and Clinical Microbiology Laboratory and leading us in the field of research.

Declarations

Conflict of interest The authors declare that they have no conflict of interest. All laboratory materials used for colistin microdilution, PFGE and molecular tests were provided free of charge from relevant manufacturers and suppliers. Biostatistician of the study is a licensed user of the package program IBM spss ver. 22.

Ethical approval The study was approved by Istanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty Ethics Committee on February 10, 2018, with an approval number of 83045809-604.01.02-A27. Since the study was based on the analysis of retrospective case data, the board did not require voluntary consent from patients or legal heirs retrospectively.

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