

Colistin Resistance in Carbapenem-Resistant *Klebsiella pneumoniae*: Laboratory Detection and Impact on Mortality

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Background. Polymyxins including colistin are an important “last-line” treatment for infections caused by carbapenem-resistant *Klebsiella pneumoniae* (CRKp). Increasing use of colistin has led to resistance to this cationic antimicrobial peptide.

Methods. A cohort nested within the Consortium on Resistance against Carbapenems in *Klebsiella pneumoniae* (CRACKLE) was constructed of patients with infection, or colonization with CRKp isolates tested for colistin susceptibility during the study period of December, 2011 to October, 2014. Reference colistin resistance determination as performed by broth macrodilution was compared to results from clinical microbiology laboratories (Etest) and to polymyxin resistance testing. Each patient was included once, at the time of their first colistin-tested CRKp positive culture. Time to 30-day in-hospital all-cause mortality was evaluated by Kaplan-Meier curves and Cox proportional hazard modeling.

Results. In 246 patients with CRKp, 13% possessed ColR CRKp. ColR was underestimated by Etest (very major error rate = 35%, major error rate = 0.4%). A variety of rep-PCR strain types were encountered in both the ColS and the ColR groups. Carbapenem resistance was mediated primarily by *bla*_{KPC-2} (46%) and *bla*_{KPC-3} (50%). ColR was associated with increased hazard for in-hospital mortality (aHR 3.48; 95% confidence interval, 1.73–6.57; *P* < .001). The plasmid-associated ColR genes, *mcr-1* and *mcr-2* were not detected in any of the ColR CRKp.

Conclusions. In this cohort, 13% of patients with CRKp presented with ColR CRKp. The apparent polyclonal nature of the isolates suggests *de novo* emergence of ColR in this cohort as the primary factor driving ColR. Importantly, mortality was increased in patients with ColR isolates.

Keywords. colistin; carbapenem-resistant Enterobacteriaceae; *Klebsiella pneumoniae*; mortality; ST258.

Carbapenem-resistant *Klebsiella pneumoniae* (CRKp) infections remain a significant challenge associated with morbidity and mortality worldwide [1, 2]. Few antimicrobials retain activity against CRKp [3]. These include aminoglycosides, tigecycline, and the recently approved ceftazidime/avibactam. In addition, polymyxins including colistin are an important treatment option [3]. After being mostly

abandoned in the 1970s, these cationic antimicrobial peptides have reemerged as a “last-line” therapeutic option for several multidrug-resistant organisms [4, 5]. In severe CRKp infections, colistin is often used in combination with other antibiotics including tigecycline, meropenem, gentamicin, or fosfomycin [5].

With the rise in consumption of colistin, cases of colistin-resistant *Klebsiella pneumoniae* carbapenemase (KPC)-producing strains are reported globally [6]. This emergence of colistin-resistant (ColR) in CRKp creates a therapeutic challenge that threatens to return clinicians and patients to a “pre-antibiotic era.” Here, data from the multicenter Consortium on Resistance against Carbapenems in *Klebsiella pneumoniae* (CRACKLE) were analyzed to determine risk factors for ColR and to compare outcomes of patients with ColR CRKp vs colistin-susceptible (ColS) CRKp.

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METHODS

Patients

CRACKLE is a prospective, multicenter, observational study of hospitalized patients with CRKp in the Great Lakes region of the United States [2, 7–9]. In this nested cohort within CRACKLE, patients were included once at the time of their first culture positive for a CRKp isolate for which in vitro colistin susceptibility testing was performed as a part of clinical care at each study site from 2011 December to 2014 October. All health systems involved in this study had approval from their respective institutional review board.

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) [10]. Bacterial identification and routine antimicrobial susceptibility testing were performed with MicroScan (Siemens Healthcare Diagnostics) or Vitek2 (bioMérieux), supplemented by GN4F Sensititre tray (Thermo Fisher) or Etest (bioMérieux), as indicated. Colistin minimum inhibitory concentration (MIC) determination was performed in clinical microbiology laboratories using Etest and was reported as “no interpretation” due to a lack of CLSI and Food and Drug Administration (FDA) breakpoints.

Colistin (sulfate salt; Sigma-Aldrich) and polymyxin B (Sigma-Aldrich) MIC determination in the central research laboratory was performed in duplicate using a broth macrodilution method in glass sterile tubes using *Escherichia coli* ATCC25922 as quality control, according to CLSI guidelines [11]. If the 2 MIC values were >1 dilution apart, further repeats were performed to determine the final MIC. The final colistin MIC thus determined was used to designate ColR vs ColS isolates. 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]. Briefly, very major errors were those in which the clinical laboratory result was susceptible and the reference method result was resistant, and major errors were defined as those in which the clinical laboratory result was resistant and the reference method result was susceptible. Very major and major error rates were calculated using the 31 ColR CRKp isolates and the 215 ColS CRKp isolates as the denominator, respectively.

Detection of carbapenemase genes and repetitive extragenic palindromic (rep)-polymerase chain reaction (PCR) strain typing was performed as previously described [2]. Briefly, PCR amplification of *bla*_{KPC}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{IMP}, and *bla*_{OXA-48} genes was conducted using validated primers and methods; amplicons were sequenced at a commercial sequencing facility (MCLAB, San Francisco, California) and analyzed [14, 15]. rep-PCR was performed using the DiversiLab Strain typing system (Bacterial

BarCodes, bioMérieux, Athens, Georgia). Isolates with ≥95% similarity were considered to be of the same rep-PCR type [16]. In addition, all ColR CRKp were tested for the presence of *mcr-1* and *mcr-2* through PCR using the following primers: *mcr-1*_F 5'-ATGATGCAGCATACTTCTGTGTGG-3'; *mcr-1*_R 5'-GTGCGGTCTTTGACTTTGTCC-3' (amplifying the complete *mcr-1* ORF according to GenBank sequence KU341381.1), *mcr-2*_IF 5'-TGTTGCTTGTGCCGATTGGA-3'; and *mcr-2*_IR 5'-AGATGGTATTGTTGGTTGCTG-3' [17].

Clinical Data

Clinical data were entered into a centralized database using the electronic medical record (EMR). The index hospitalization was recorded as the first hospital stay within the study period during which the CRKp was obtained and tested for colistin susceptibility. CRKp infections were defined by the standardized criteria described previously [2]. Hospital acquisition was defined as timing of the first positive culture more than 2 days into the admission. Critical illness was determined as a Pitt bacteremia score ≥4 points on the day of the index culture [18]. The Charlson comorbidity index was calculated, as described elsewhere [19].

Statistics

Differences between groups were analyzed using Wilcoxon rank sum for continuous variables. Fisher exact and Pearson testing were used for categorical variables where appropriate. Kaplan-Meier curves were constructed to compare time to 30-day in-hospital mortality between groups. Adjusted hazard ratios (aHRs) were calculated using Cox proportional hazards modeling on time to 30-day in-hospital mortality. In the Cox model, all variables were considered that were associated at the $P < .1$ level with the outcome, followed by backward selection. P values ≤ .05 were considered statistically significant. JMP 10.0.1 software (SAS, Inc, Cary, North Carolina) was used for all analyses.

RESULTS

Colistin Susceptibility Testing

A total of 522 unique patients were included in the CRACKLE study during the study period. Of these 522 patients, 246 (47%) had at least 1 isolate that was shipped to the central research laboratory that had been tested for colistin susceptibility as part of routine clinical care. These 246 patients were included in the study. Colistin resistance per research laboratory determination (“ColR”) was detected in the CRKp isolates for 31 (13%) patients compared to 21 (9%) per clinical laboratory testing. Of note, additional colistin MIC testing beyond duplicates was required in 25 (10%) isolates; the clinical laboratory reported all of these isolates as susceptible. In addition, 25 (10%) of 240 tested isolates were in vitro resistant to polymyxin B as determined by the central research laboratory. In the remaining 6 (2%) isolates, a

definitive polymyxin B MIC could not be determined in spite of repetitive testing, due to the observance of “skipped wells” and trailing endpoints.

The distributions of colistin MIC as determined by the central research laboratory compared to the clinical microbiology laboratories and compared to polymyxin B MIC are shown in Figure 1. Testing in the research laboratory resulted in a different interpretation in 12/246 (5%) isolates (Figure 1). The very major error rate was 35% (11/31), and the major error rate was 0.4% (1/215).

Clinical Characteristics

Characteristics of patients with ColR vs ColS CRKp were similar (Table 1). Overall, 111 (45%) patients met criteria for CRKp infection. The anatomic sources of CRKp were urine (59%), blood (18%), respiratory system (11%), wound (5%), and others (7%). Remarkably, time from admission to first positive culture was shorter in patients in the ColR vs ColS group; at a median of 0 days (interquartile range [IQR], 0–3 days) in the ColR group vs 1 day (IQR, 0–9 days) in the ColS group ($P = .01$). The total length of stay (LOS) was also shorter for patients with ColR isolates; a median of 8 days (IQR, 5–12 days) compared to 13 days (IQR, 7–26 days) in the ColS group ($P = .01$). When only patients who survived their hospital stay were analyzed, this difference in LOS was similar, albeit no longer significant (median 8 vs 12 days; $P = .08$). Finally, a marginal association was observed with prehospitalization origin; patients with ColR CRKp were more likely to be admitted from home or a long-term acute care facility (LTAC), whereas patients with ColS CRKp were more likely to be admitted from a skilled nursing facility (SNF) or to be transferred from another hospital ($P = .05$). No specific SNF or LTAC appeared to be associated with ColR; 12 patients with ColR isolates were admitted from 4 LTACs and 5 SNFs.

Previous Antibiotic Exposure

Antibiotic exposures in the 14 days preceding the first positive culture were analyzed (Table 2). In 82 (33%) patients, antibiotic use was not documented. In the remaining 164 (67%), vancomycin was the single most common drug exposure and was given to 80 (33%) patients. Twenty-seven (11%) patients received more than 3 classes of antibiotics in the 14-day period before the positive culture. Exposure to fluoroquinolones was less common in the ColR group compared to the ColS group. Otherwise, antibiotic exposures in this time period were not different between patients with or without ColR CRKp.

Documented previous colistin use in the 14 days leading up to the day of first positive culture was uncommon in the cohort as a whole, and patients in the ColR group did not receive colistin during this limited time period. Colistin exposure data beyond the 14-day period were available for 27 patients with ColR isolates, based on EMR of the healthcare system of the index hospitalization. In total, 6/27 (22%) patients were found to have previous colistin exposure at any time prior to first positive culture with a ColR CRKp.

Microbiology

The susceptibilities for antibiotics other than colistin were determined as clinically indicated by clinical microbiology laboratories (Figure 2). ColR CRKp were significantly less likely to be amikacin susceptible; 8/15 (53%) of tested ColR CRKp were amikacin susceptible compared to 104/126 (83%) of tested ColS CRKp ($P = .02$). In contrast, ColR CRKp were more likely to be gentamicin susceptible; 21/31 (68%) of tested ColR CRKp were gentamicin susceptible compared to 97/212 (46%) of tested ColS CRKp ($P = .03$). Furthermore, ColR isolates were also more likely to be tigecycline nonsusceptible; 13/21 (62%) of tested ColR CRKp were tigecycline susceptible compared to 138/168 (82%) of tested ColS CRKp ($P = .04$).

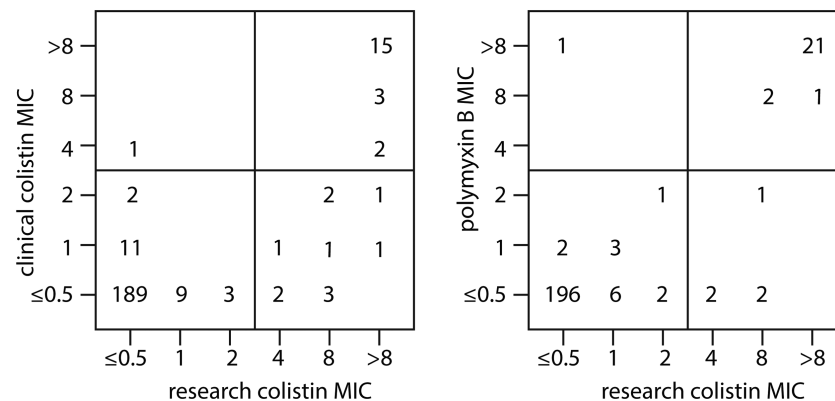


Figure 1. The numbers of isolates that correspond to each minimum inhibitory concentration (MIC) value for the MIC as reported by the clinical microbiology laboratories (“clinical colistin MIC”) and the centralized research laboratory (“research colistin MIC”), as well as the polymyxin B MIC are shown.

Table 1. Clinical Characteristics

Characteristic	All	Colistin Resistant	Colistin Susceptible	PValue ^a
N	246	31	215	
Age in years, median (IQR)	67 (54–77)	62 (53–73)	67 (54–78)	.43
Female	139 (57)	20 (65)	119 (55)	.44
Race/Ethnicity				.22
White	135 (55)	13 (42)	122 (57)	
Black	95 (39)	16 (52)	79 (37)	
Hispanic	7 (3)	0	7 (3)	
Other	9 (4)	2 (6)	7 (3)	
Charlson comorbidity index, median (IQR)	3 (2–5)	3 (2–4)	3 (2–5)	.78
Diabetes mellitus	118 (48)	15 (48)	103 (48)	1.0
Renal failure ^b	75 (30)	7 (23)	68 (32)	.40
Heart disease	121 (49)	14 (45)	107 (50)	.70
Chronic obstructive pulmonary disease	62 (25)	8 (26)	54 (25)	1.0
Malignancy	41 (17)	8 (26)	33 (15)	.19
Infection	111 (45)	13 (42)	98 (46)	.85
Source				.22
Urine	144 (59)	16 (52)	128 (60)	
Blood	45 (18)	5 (16)	40 (19)	
Respiratory system	28 (11)	4 (13)	24 (11)	
Wound	13 (5)	1 (3)	12 (6)	
Other	16 (7)	5 (16)	11 (5)	
Days to first positive culture, median (IQR)	1 (0–7)	0 (0–3)	1 (0–9)	.01
Hospital acquisition	115 (47)	10 (32)	105 (49)	.12
Origin				.05
Home	90 (37)	14 (45)	76 (35)	
Skilled nursing facility	76 (31)	6 (19)	70 (33)	
Hospital transfer	59 (24)	5 (16)	54 (25)	
Long-term acute care	21 (9)	6 (19)	15 (7)	
Critical illness ^c	86 (35)	7 (23)	79 (37)	.16
Length of stay in days, median (IQR)	12 (6–24)	8 (5–12)	13 (7–26)	.01

All data expressed as n (%), unless otherwise indicated.

Abbreviation: IQR, interquartile range.

^aUnivariable relationship between variable of interest and colistin resistance.

^bRenal failure defined as creatinine >2 mg/dL on admission.

^cCritical illness defined as Pitt bacteremia score ≥4 at the time of index culture.

Overall, a nonclonal distribution was observed. rep-PCR suggested that most strains belonged to clades ST258A (n = 76, 31%) and ST258B (n = 102, 41%). The remaining 68 (28%) isolates belonged to 28 other rep-PCR types. Likewise in the ColR group, a nonclonal pattern was observed (Figure 3). rep-PCR types corresponding to clades ST258A and ST258B together accounted for 77% of ColR isolates.

The presence of carbapenemase genes was confirmed in 238 (97%) isolates. The carbapenemase genes *bla*_{KPC-2} and *bla*_{KPC-3} were the most common and were found in 113 (46%) and 124 (50%) of CRKp isolates, respectively. The distribution of carbapenemase genes did not differ between ColR and ColS CRKp. Of note, *bla*_{NDM-1} was found in only 1 isolate. This

Table 2. Antibiotic Exposures

Variable	All (n = 246)	Colistin Resistant (n = 31)	Colistin Susceptible (n = 215)	PValue
Classes of antibiotics				.68
None	82 (33)	10 (32)	72 (33)	
1	51 (21)	9 (29)	42 (20)	
2	51 (21)	5 (16)	46 (21)	
3	35 (14)	5 (16)	30 (14)	
>3	27 (11)	2 (6)	25 (12)	
Tigecycline	7 (3)	2 (6)	5 (2)	.22
Carbapenem	36 (15)	4 (13)	32 (15)	1.0
Fluoroquinolone	44 (18)	1 (3)	43 (20)	.02
Colistin	2 (1)	0	2 (1)	1.0
Vancomycin	80 (33)	8 (26)	72 (33)	.54
β-lactam/β-lactamase inhibitor	52 (21)	7 (23)	45 (21)	.82
Cephalosporin	25 (10)	5 (16)	20 (9)	.22
Aminoglycoside	14 (6)	2 (6)	12 (6)	.69
Daptomycin	10 (4)	2 (6)	8 (4)	.37
Metronidazole	19 (8)	4 (13)	15 (7)	.27
Other	90 (37)	11 (35)	79 (37)	1.0

Shown is the documented exposure to antibiotics in 14 days prior to first positive culture. All data expressed as n (%).

ColS isolate was extensively described previously using whole genome sequencing [2]. In the remaining 8 (3%) isolates, carbapenemase genes were not detected. The plasmid-mediated colistin resistance genes, *mcr-1* and *mcr-2*, were not detected in any of the ColR CRKp.

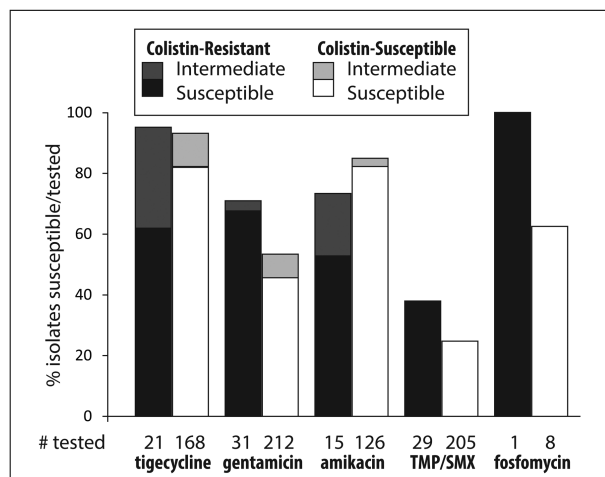


Figure 2. The percentages of susceptible and intermediate isolates for each drug are shown separately for colistin-resistant (ColR) and colistin-susceptible (ColS) carbapenem-resistant *Klebsiella pneumoniae* (CRKp). Underneath each bar is the number of CRKp tested for each drug in the ColR and ColS groups. The black and white bars represent the percentage of susceptible/tested CRKp isolates in the ColR and ColS groups, respectively. The dark-gray and light-gray bars on top of the black and white bars represent the percentage of intermediate/tested CRKp isolates in the ColR and ColS groups, respectively. Abbreviation: TMP/SMX, trimethoprim/sulfamethoxazole.

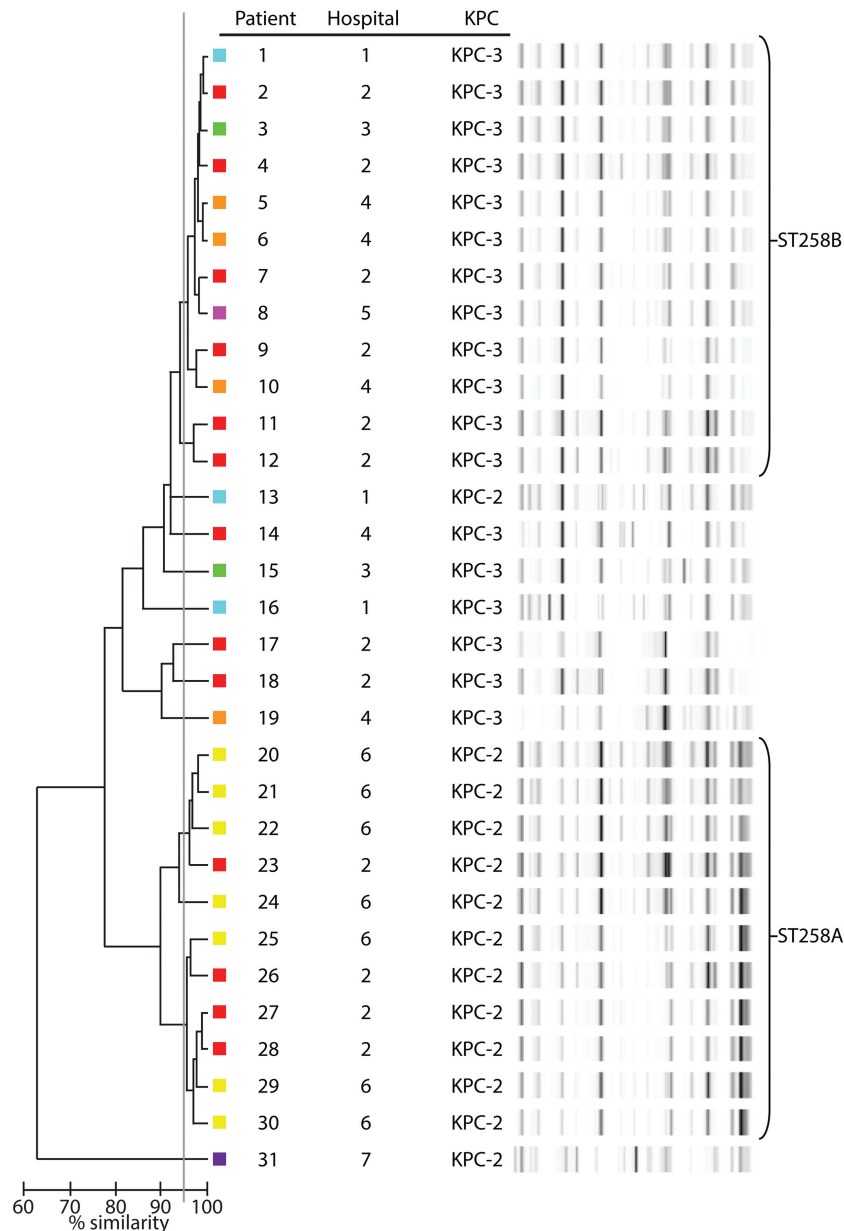


Figure 3. Repetitive extragenic palindromic–polymerase chain reaction (rep-PCR) dendrogram of colistin-resistant carbapenem-resistant *Klebsiella pneumoniae* isolates. A $\geq 95\%$ similarity cutoff (gray line) was used to consider isolates of the same rep-PCR type. Colored boxes next to each strain represent the various hospitals from which isolates were recovered. Abbreviation: KPC, *klebsiella pneumoniae* carbapenemase.

Treatment and Mortality

In the first 7 days after the first positive culture, 143 (58%) patients received directed treatment. Treatment regimens used in these 143 patients are summarized in Table 3. Treatment with colistin was uncommon; colistin, either alone or in combination with other antibiotics, was given to 31 (22%) of treated patients. Colistin treatment was given to 30 of 127 (24%) treated patients in the ColS group compared to 1/16 (6%) treated patients in the ColR group. Other treatment regimens were similar between the 2 groups. The most commonly used antibiotics were aminoglycosides and tigecycline. Aminoglycosides were

given to 72 (50%) of treated patients; 36 (25%) patients received only aminoglycosides in the first 7 days. Similarly, 63 (44%) of treated patients received tigecycline, of whom 21 (15%) received tigecycline monotherapy in the first 7 days.

We evaluated time to all-cause in-hospital mortality, censored at 30 days (Figure 4). In univariable Kaplan-Meier survival curves, patients with ColR CRKp were at increased hazard of 30-day mortality ($P < .0001$). After adjustment, the aHR of ColR vs ColS was 3.48 (95% confidence interval [CI] 1.73–6.57; $P < .001$). Other variables that impacted survival included age, pre-hospitalization origin, and anatomical source of the

Table 3. Treatment Characteristics

Characteristic	All	Colistin Resistant	Colistin Susceptible
n	143	16	127
Any colistin in first 7 days	31 (22)	1 (6)	30 (24)
One antibiotic in first 7 days ^a	90 (63)	10 (63)	80 (63)
Aminoglycoside	36 (25)	5 (31)	31 (24)
Colistin	10 (7)	1 (6)	9 (7)
Tigecycline	21 (15)	3 (19)	18 (14)
Trimethoprim/sulfamethoxazole	13 (9)	0	13 (10)
fosfomycin	10 (7)	1 (6)	9 (7)
>1 antibiotic in first 7 days	53 (37)	6 (38)	47 (37)
2 antibiotics	38 (27)	5 (31)	33 (26)
3 antibiotics	13 (9)	1 (6)	12 (9)
4 antibiotics	2 (1)	0	2 (2)

Treatment is shown in the subset of patients who received at least 1 antibiotic with potential in vitro anti-carbapenem-resistant *Klebsiella pneumoniae* (CRKp) activity in the first 7 days after first positive CRKp culture.

^aOnly antibiotics with potential in vitro anti-CRKp activity were included.

positive CRKp culture (Table 4). Treatment variables and infection vs colonization status were not associated with mortality, and inclusion of these into the model did not significantly alter the association between colistin and mortality. Of note, when the colistin resistance as determined by the clinical microbiology laboratory was entered into this model instead of ColR as determined by the research laboratory, this was still associated with increased mortality but to a lesser extent (aHR, 2.78; 95% CI, 1.22–5.71; $P = .02$; data not shown).

DISCUSSION

In this nested cohort of patients from CRACKLE—a prospective, ongoing, multicenter evaluation of hospitalized patients

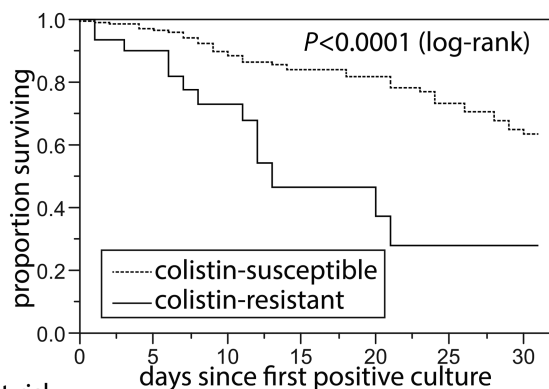


Figure 4. Kaplan-Meier curve showing the 30-day in-hospital survival for patients with colistin-resistant carbapenem-resistant *Klebsiella pneumoniae* (CRKp) as compared to colistin-susceptible CRKp. Patients were censored at the time of hospital discharge.

Table 4. Cox Proportional Hazards Model on Time to 30-Day In-Hospital Mortality

Variable	Adjusted Hazard Ratio	95% Confidence Interval	P Value
Colistin resistance	3.48	1.73–6.57	< .001
Age (by decade)	1.24	1.03–1.53	.02
Origin			< .01
Home (reference)	—	—	
Skilled nursing facility	2.52	1.12–6.03	
Hospital transfer	2.64	1.18–6.35	
Long-term acute care	5.18	2.17–12.82	
Source			< .01
Blood (reference)	—	—	
Urine	0.35	0.19–0.66	
Other	0.29	0.13–0.60	

with carbapenem-resistant Enterobacteriaceae (CRE)—a ColR rate of 13% was observed in CRKp isolates. This rate was underestimated by testing as performed by clinical microbiology laboratories. In addition, even testing in optimal research conditions resulted in the need for repeat colistin MIC testing in 10% of isolates. Furthermore, polymyxin B MIC testing was also fraught with difficulties; a definitive polymyxin B MIC could not be determined in 6 isolates, even with repetitive testing. The difficulty in determining colistin MIC has been previously reported by Hindler et al [20]. Broth macrodilution, as used here, was found to be the most reliable [20]. The American Society for Microbiology recently released a white paper on the issue of colistin testing, recommending that “laboratories that choose to test for colistin susceptibility should validate a broth microdilution method and report MIC values only” [21]. Clearly, the absence of FDA breakpoints and the resulting impossibility of an FDA-approved test for colistin susceptibility remains a major issue.

The association between ColR and mortality was also seen in an Italian case-control study on CRKp bloodstream infections [22]. In that study 30-day all-cause mortality rates were 51% in the ColR group (n = 142) vs 39% in the ColS group (n = 284; $P = .02$). The increased mortality may be directly related to decreased colistin susceptibility of the organism. Alternatively, the observed mortality differences may be secondary to either variance in baseline characteristics of patients with ColR vs ColS CRKp or to an alteration in the virulence of the pathogen [23]. Regardless of causality, it is clear that patients with ColR CRKp are at increased risk of hospital mortality.

We observed that—similar to patients with tigecycline-resistant CRKp—isolation of ColR CRKp tended to occur earlier during hospitalization compared to ColS CRKp [7]. Our findings suggest that the introduction of ColR isolates may be present on admission to acute care hospitals. In the majority of patients, colistin exposure prior to the positive culture was not documented. This may be explained in a number of ways. First, some patients may have had unrecorded colistin exposure

at nonstudy hospitals. The intuitive link between colistin exposure and ColR has been previously documented for CRKp and carbapenem-resistant *Acinetobacter baumannii* (CRAB) [4, 22]. Second, patients with ColR CRKp without colistin exposure may have received ColR CRKp from either another person exposed to colistin or potentially from a food source. The latter option would appear less likely as no such food-related transmission has been reported in the United States for *K. pneumoniae*. In contrast, person-to-person transmission of ColR CRKp without further colistin exposure of subsequently infected patients was shown to be an important mechanism of spread in an outbreak-related report [24]. The lack of clonality in ColR CRKp described in that report argues against a single clonal outbreak but obviously does not rule out multiple person-to-person transmission events.

Previously we described various chromosomally mediated mechanisms of ColR for a subset of isolates in this cohort [25]. Of concern, 2 recently described plasmid-mediated, readily transmissible ColR mechanisms—*mcr-1* and *mcr-2*—are being recognized in Enterobacteriaceae from animals and humans around the world [17, 26]. Even though *mcr-1* has already been reported in the United States, this gene was not detected in any of the isolates described here [27, 28]. Reproducibility issues in polymyxin/colistin MIC testing, as well as variability between testing methods illustrated here and in other reports, may complicate tracking of *mcr-1*- and *mcr-2*-carrying isolates [20]. If screening of *mcr* carriage is only performed on isolates based on colistin MIC by Etest, the most common method used by clinical labs, the spread of *mcr* is likely to be underestimated.

There are a number of limitations to this work. We purposefully selected isolates in which colistin susceptibility testing was performed by the clinical microbiology laboratories as clinically indicated, and patients with CRKp isolates that were not tested are not included in this study. Therefore, our estimate of 13% ColR at first test may not necessarily be representative of all patients with CRKp. Furthermore, follow-up for patients in this observational study is limited to the duration of hospitalization. Consequently, we cannot comment on long-term outcomes. Another potential limitation of our work is that we included both patients who met standardized criteria for infection as well as those who did not. Nevertheless, standardized criteria have limitations in distinguishing patients with infection from those who are colonized. In addition, patients who do not meet criteria for infection often receive antibiotic treatment and are an important source of transmission. Finally, our study population was predominantly derived from the Great Lakes region of the United States. Therefore, these data may not accurately reflect incidence of ColR in other CRKp in the United States. In a study performed by the Centers for Disease Control and Prevention in 2012–2013 in 7 US communities, 12 CRE isolates were tested for colistin susceptibility. Nine of these were reported to be non-susceptible, based on testing performed in clinical laboratories

[29]. Additional data from other US regions are expected from ongoing surveillance programs and from the recently started national expansion of CRACKLE. Additionally, there are important differences in ColR rates in CRE isolates outside of the United States as well [17, 26].

In summary, we observed a 13% ColR rate in this multicenter, observational cohort of hospitalized patients with CRKp in US hospitals. Colistin in vitro susceptibility testing is characterized by reproducibility issues. Patients with ColR CRKp were at higher risk for mortality. They also tended to present earlier during admission. This, in combination with the polyclonality of the observed ColR CRKp isolates, suggests that direct patient-to-patient transmission of CRKp isolates within short-term acute care hospitals is unlikely to be the primary mechanism driving resistance rates. Alternative, not mutually exclusive explanations include within-patient development of ColR and transmission in healthcare settings outside of short-term acute care hospitals, such as LTACs or SNFs.

NOTES

Disclaimer. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

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