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Doripenem MICs and *ompK36* Porin Genotypes of Sequence Type 258, KPC-Producing *Klebsiella pneumoniae* May Predict Responses to Carbapenem-Colistin Combination Therapy among Patients with Bacteremia

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Treatment failures of a carbapenem-colistin regimen among patients with bacteremia due to sequence type 258 (ST258), KPC-2-producing *Klebsiella pneumoniae* were significantly more likely if both agents were inactive *in vitro*, as defined by a colistin MIC of >2 μg/ml and the presence of either a major *ompK36* porin mutation (guanine and alanine insertions at amino acids 134 and 135 [ins aa 134–135 GD], IS*5* promoter insertion [$P = 0.007$]) or a doripenem MIC of >8 μg/ml ($P = 0.01$). Major *ompK36* mutations among KPC-*K. pneumoniae* strains are important determinants of carbapenem-colistin responses *in vitro* and *in vivo*.

Carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp) strains have emerged worldwide, causing infections associated with significant mortality (1–3). Carbapenem resistance among strains in the United States is typically mediated by production of carbapenem-hydrolyzing enzymes, in particular *Klebsiella pneumoniae* carbapenemases (KPCs). Additional mechanisms include production of beta-lactamases and alterations in outer membrane porins (OMPs) (2). At our center, sequence type 258 (ST258), KPC-2-producing strains that harbor TEM-1 and SHV-12 beta-lactamases predominate (2). ST258, KPC-2-producing strains carry a mutant *ompK35* gene that encodes a truncated porin (amino acid [aa] 89 STOP) and exhibit one of several *ompK36* porin genotypes. The most active antimicrobial regimen against our strains *in vitro* is a carbapenem-colistin combination (4). However, two major *ompK36* mutations (guanine and alanine insertions at aa 134 and 135 [ins aa134–135 GD] and an IS*5* insertion sequence within the promoter) are associated with high-level carbapenem resistance (MIC, >8 μg/ml) and attenuated time-kill responses to carbapenem-colistin (4, 5).

Although retrospective studies have reported improved outcomes among patients with CR-Kp bacteremia treated with combination therapy rather than single agents (1, 6, 7), the response to carbapenem-based combination therapy was not consistent. To date, it is unclear which patients would benefit from this combination therapy. We hypothesized that carbapenem-colistin treatment failures are at least partially explained by the presence of major *ompK36* mutations. The objective of this study was to evaluate the association between *ompK36* genotypes, doripenem MICs, and responses to carbapenem-colistin therapy among patients with CR-Kp bacteremia.

We conducted a retrospective study of patients with CR-Kp bacteremia at our center between February 2010 and May 2013. CR-Kp was defined by nonsusceptibility to a carbapenem and all third-generation cephalosporins (8). Patients with bacteremia initially treated with carbapenem-colistin (colistimethate) for >3 days were included. For patients with normal renal function, standardized doses of colistin (5 mg/kg body weight loading, followed by 5 mg/kg/day divided into 3 doses) and doripenem (our formulary agent; 1 g every 8 h, infused over 3 h) were recommended. Among patients with renal impairment, doses were adjusted based on published recommendations (9). "Cure" was defined as resolution of symptoms and sterilization of blood cultures within 7 days. "Treatment failure" was defined as death within 7 days, persistent signs and symptoms of CR-Kp infection despite ≥7 days of therapy, recurrence of CR-Kp infection within 90 days, or breakthrough CR-Kp bacteremia while receiving carbapenem-colistin. Strains were tested for multilocus sequence type, KPC variant, beta-lactamases, and porin genotypes by standard methods (2). Doripenem and colistin MICs were determined using CLSI methods, and resistance was defined by MICs of >2 μg/ml (10). Based on our previously published *in vitro* studies and relevant pharmacokinetic-pharmacodynamic (PK-PD) data, carbapenems were considered inactive if the MIC of the agent used in treatment was >8 μg/ml (5–7). Comparisons between groups were made by Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. Significance was defined as $P \leq 0.05$ (two tailed).

Twenty-seven patients with CR-Kp bacteremia who received

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TABLE 1 Patient demographics, clinical characteristics, and outcomes of CR-Kp bloodstream infections[a]

Patient	Age, yr (sex)	Underlying disease(s)	APACHE II score[b]	Time to BSI, days[c]	Portal of entry	Source control	Duration of BSI, days[d]	Concomitant CR-Kp infection at time of BSI	Time to initiation of therapy, h[e]	No. of days of therapy	MIC (μg/ml)		*ompK*36 genotype	Treatment outcome
											Colistin	Doripenem		
1	72 (M)	Metastatic pancreatic carcinoma	18	2	Abdominal	Percutaneous drainage of bile duct (day 3)	5	None	81	17	0.25	8[f]	WT	Cure
2	75 (F)	ESRD, CHF, CAD	16	33	Abdominal	None	1	None	47	13	0.25	8[f]	WT	Cure
3	48 (M)	Primary sclerosing cholangitis	7	1	Abdominal	Cholecystectomy (day 18)	1	None	65	14	0.25	16	WT	Cure
4	68 (M)	Pancreatic cancer, cholecystitis	12	1	Abdominal	Drainage via cholecystostomy tube (day 1), subtotal cholecystectomy (day 18)	2	IAI	62	14	0.25	8	WT	Cure
5	50 (M)	Kidney and liver transplant	16	51	Abdominal	None	1	IAI	26	19	0.25	8	aa 84–87 (NNTE) del	Cure
6	63 (F)	Liver transplant	17	3	Abdominal	Percutaneous drainage of abscess (day 2)	48	IAA	92	75	0.5	8	WT	Failure due to persistent then recurrent BSI (day 90)
7	37 (F)	Caroli disease	7	1	Abdominal	None	1	None	77	19	0.25	16	WT	Failure due to persistent IAI requiring liver resection and transplantation
8	54 (M)	Liver transplant	14	122	Vascular catheter	Catheter removal (day 2)	1	PNA	Breakthrough BSI	44	64	16	WT	Failure due to breakthrough BSI
9	56 (M)	Liver transplant	20	21	Urine	None	2	Cystitis	28	16	0.5	128	ins aa 134–135 DG	Cure
10	55 (M)	Polycythemia	33	44	Respiratory tract	Chest tube insertion (day 6)	1	PNA, empyema	Breakthrough BSI	19	0.25	128	ins aa 134–135 DG	Failure due to breakthrough BSI
11	65 (F)	Kidney transplant	24	81	Respiratory tract	None	1	PNA	Breakthrough BSI	59	0.5	128	ins aa 134–135 DG	Failure due to breakthrough BSI
12	44 (M)	Multivisceral transplant	21	22	Vascular catheter	Catheter exchange (day 1), removal of TDC (day 2)	1	None	Breakthrough BSI	54	64	64	ins aa 134–135 DG	Failure due to breakthrough BSI and new site of infection (PNA, day 23)
13	55 (M)	Polymyositis	30	102	Abdominal	Surgical debridement and abdominal washout (days 3 and 5)	1	IAI	76	4	0.25	64	ins aa 134–135 DG	Failure due to death (day 7)
14	57 (M)	ESLD	30	58	Respiratory tract	None	2	PNA	15	4	0.25	64	ins aa 134–135 DG	Failure due to death (day 4)
15	60 (M)	DM, necrotizing pancreatitis	28	24	Abdominal	Abdominal washout (days 1, 9, and 11), surgical debridement (day 13)	27	IAI	Breakthrough BSI	29	0.5	128	ins aa 134–135 DG	Failure due to breakthrough and persistent BSI, death (day 28)

16	58 (F)	Liver transplant	16	9	Abdominal	Percutaneous drain of abscess (day 1), abdominal washout (days 3, 8, 19, 23, 33, and 39)	9	IAI	51	141 (gentamicin, 137)[g]	0.5	64	ins aa 134–135 DG	Failure due to persistent BSI, then recurrent BSI (days 46 and 87), new site of infection (PNA, day 39)
17	61 (M)	Liver transplant	14	30	Vascular catheter	Catheter removed (day 3)	2	None	67	10	0.25	128	ins aa 134–135 DG	Failure due to recurrent infection (PNA, day 31)
18	62 (M)	Kidney transplant	23	6	Abdominal/ urinary	Removal of ureteral stent (day 3), nephrectomy (day 21)	42	Pyelonephritis	53	35	16	16	ins aa 134–135 DG	Failure due to persistent BSI
19	26 (M)	Gallstone pancreatitis	9	36	Abdominal	Drainage of gallbladder and gall stone removal (day 2)	1	IAI	53	38	0.25	128	IS*5*	Failure due to recurrent IAI (day 16)
20	59 (M)	DM, CRF	10	1	Abdominal	None	9	IAI	69	5	0.5	128	IS*5*	Failure due to persistent BSI and death (day 9)

[a] Abbreviations: BSI, bloodstream infection (bacteremia); CAD, coronary artery disease; CHF, congestive heart failure; CRF, chronic renal failure; DM, diabetes mellitus; ESLD, end-stage liver disease; F, female; HIV, human immunodeficiency virus; IAI, intra-abdominal infection; IAA, intra-abdominal abscess; M, male; PNA, pneumonia; TDC, tunneled dialysis catheter; WT, wild type.

[b] At the onset of bloodstream infection.

[c] Time from hospital admission to positive blood culture.

[d] Days of positive blood cultures.

[e] Time from collection of blood culture to first dose of combination therapy.

[f] Patients 1 and 2 were treated with meropenem. The meropenem MIC was 8 μg/ml in each case. All other patients were treated with doripenem.

[g] Gentamicin was added to the doripenem-colistin combination due to persistent infection.

TABLE 2 Rates of clinical cure by number of active agents in the treatment combination

Definition of resistance	Regimen	Rate of clinical cure, % (no. cured/total)	*P* value[a]
Doripenem MIC of >8 μg/ml and colistin MIC of >2 μg/ml	Both agents active	80 (4/5)	0.01
	1 agent active	17 (2/12)	
	No agent active	0 (0/3)	
Major *ompK36* mutation and colistin MIC of >2 μg/ml	Both agents active	71 (5/7)	0.007
	1 agent active	9 (1/11)	
	No agent active	0 (0/2)	

[a] Comparing both agents active versus one or no agent active.

TABLE 3 Factors associated with carbapenem-colistin treatment failure

Factor	Value for patients with indicated outcome		*P* value
	Cure ($n = 6$)	Failure ($n = 14$)	
Solid organ transplant recipient, no. (%)	2 (33)	9 (64)	0.36
Renal replacement therapy, no. (%)	1 (17)	6 (43)	0.35
Residence in intensive care unit, no. (%)	2 (33)	11 (79)	0.12
Median APACHE II score (range)	16 (7–20)	19 (7–33)	0.28
Median time from admission to BSI,[a] days (range)	12 (1–51)	27 (1–122)	0.23
Concomitant CR-Kp infection, no. (%)	3 (50)	11 (79)	0.30
Median time to initiation of combination therapy, h (range)[b]	55 (26–81)	52 (15–92)	0.71
Appropriate colistin dosing, no. (%)[c]	4 (67)	9 (64)	1.00
Major *ompK36* mutation, no. (%)	1 (17)	11 (79)	0.02

[a] BSI, bloodstream infection.
[b] Excludes patients with breakthrough bacteremia.
[c] Colistin dosing regimens were considered to be appropriate if loading doses were given and maintenance doses were prescribed according to institutional guidelines and published recommendations (9).

carbapenem-colistin therapy were identified. Seven patients were excluded due to receipt of a third agent ($n = 4$ [all received gentamicin]), death within 3 days ($n = 2$), or initiation of comfort measures only ($n = 1$). Twenty patients with CR-Kp bacteremia fulfilled inclusion criteria (Table 1). The median colistin and doripenem MICs were 0.25 and 64 μg/ml, respectively. All CR-Kp strains were ST258 strains that carried bla_{KPC-2}, bla_{TEM1}, bla_{SHV-12}, and mutant gene *ompK35* (aa 89 STOP). Sequence analysis revealed four *ompK36* genotypes, including ins aa 134–135 GD ($n = 10$), wild type ($n = 7$), IS*5* promoter insertions (IS*5*; $n = 2$), and an asparagine-asparagine-threonine-glutamic acid (NNTE) deletion at aa 84 to 87 ($n = 1$), which is a minor mutation that does not impact carbapenem-colistin responses *in vitro* (5). Median doripenem MICs were higher against major *ompK36* mutant strains than wild-type or minor mutant strains (128 versus 16 μg/ml; $P = 0.002$).

Ninety percent (18/20) of patients received doripenem, and 10% (2/20) received meropenem. Seventy percent (14/20) of patients experienced treatment failures; the 28-day mortality rate was 20% (4/20). All three patients infected with colistin-resistant strains (MIC, >2 μg/ml) failed treatment. Eighty-six percent (12/14) and 33% (2/6) of patients infected with strains that exhibited doripenem MICs of >8 μg/ml and ≤8 μg/ml, respectively, experienced treatment failure ($P = 0.04$). Ninety-two percent (11/12) of patients infected with strains harboring major *ompK36* mutations failed therapy compared to 38% (3/8) of patients infected with wild-type or minor *ompK36* mutant strains ($P = 0.02$). Clinical cures occurred in 80% (4/5) of patients who received two active agents (as defined by MICs), compared to 13% (2/15) of patients who received one or no active agent ($P = 0.01$). If carbapenem resistance was defined by the presence of a major *ompK36* mutation, cures occurred in 71% (5/7) of patients who received two active agents compared to 8% (1/13) who received one or no active agent (Table 2 [$P = 0.007$]). No other clinical factors were significantly associated with treatment failure (Table 3). The four patients who died within 28 days were infected with colistin-susceptible, major *ompK36* mutant strains that exhibited doripenem MICs of ≥64 μg/ml (ins aa 134–135 GD, $n = 3$; IS*5*, $n = 1$).

To our knowledge, this is the first study to demonstrate a correlation between molecular mechanisms of carbapenem resistance and outcomes of combination antimicrobial therapy among patients with CR-Kp bacteremia. The presence of a major *ompK36* mutation was a highly sensitive marker for a doripenem MIC of >8 μg/ml and accurately predicted carbapenem-colistin treatment failures. Moreover, carbapenem-colistin therapy was significantly more likely to be effective when both agents were active *in vitro*, as defined by colistin MICs of ≤2 μg/ml and the presence of either a major *ompK36* mutation or a doripenem MIC of ≤8 μg/ml. Our clinical experience supports *in vitro* data from our center, which demonstrated that major *ompK36* mutations and a doripenem MIC of >8 μg/ml predicted a lack of KPC-Kp responsiveness to doripenem-colistin during time-kill assays (5).

Previous studies reported decreased mortality among patients with carbapenemase-producing *K. pneumoniae* bacteremia who were treated with carbapenem-containing combinations (1, 6, 7). In two of these studies, survival was higher if carbapenem MICs were ≤8 μg/ml (6, 7). The mortality rate in our study was only 20%, but all patients who died were infected with major *ompK36* mutant strains that exhibited extremely high-level doripenem resistance. Our study differs from earlier reports by focusing specifically on colistin as the second agent in combination and by linking results to underlying mechanisms of carbapenem resistance. Positive interactions between carbapenems and colistin are consistent with a model in which membrane permeabilization by the latter facilitates increased access of the former to target sites, thereby overcoming carbapenemase hydrolysis (11). Major *ompK36* mutations constrict the porin channel (ins aa 134–135 GD mutations) or attenuate gene expression and porin levels (IS*5* promoter insertions) (12).

Our findings highlight the clinical importance of quantifying carbapenem MICs beyond current CLSI resistance breakpoints (doripenem, imipenem, and meropenem MICs of >2 μg/ml; ertapenem MIC of >1 μg/ml). Modeling data indicate that high-dose and prolonged-infusion carbapenem regimens, as recommended at our center, can increase the probability of achieving serum PK-PD targets of free-drug concentrations above the MIC ($fT > MIC$) for ≥35% of the dosing interval for inhibition of resistant *Enterobacteriaceae* if MICs are ≤8 μg/ml (13, 14). Along these lines, emerging clinical data suggest that optimized carbapenem dosing can successfully treat at least some infections due to *Enterobacteriaceae* with lower-level resistance (6, 7). Our study design precludes any conclusions about the benefits of carbap-

enem-colistin therapy over carbapenem monotherapy or definitive assessments of colistin monotherapy. It is notable that treatment failures were observed among the overwhelming majority of patients who received a regimen in which only colistin was active. A major shortcoming of many clinical studies has been the use of suboptimal colistin regimens, which confounds interpretations of the drug's effectiveness. However, 70% (7/10) of treatment failures among our patients receiving colistin as the sole active agent occurred despite recommended loading and maintenance doses (9). The poor clinical responses are in keeping with our previous *in vitro* time-kill data showing that colistin was merely bacteriostatic against colistin-susceptible KPC-Kp strains at concentrations achievable in human serum (5).

There are several limitations to this study. The retrospective design limited us to existing clinical data. It is possible that our experience was skewed by clinician biases in choosing a carbapenem-colistin regimen to treat these particular patients. Since all except two patients received doripenem, we cannot comment about other carbapenems. Indeed, it is important to acknowledge that our clinical experience may have differed if meropenem or another carbapenem was used instead of doripenem. Three patients infected with wild-type or minor mutant *ompK36* strains exhibited doripenem MICs of 16 μg/ml; two of these patients failed treatment. Therefore, we cannot comment on the significance of discrepancies between genotypic and phenotypic definitions of carbapenem inactivity. The validity of our definitions requires future studies. Finally, our results may not be relevant at all centers since our sample size was small and strain resistance phenotypes and genotypes may differ. Nevertheless, our findings are likely to be broadly representative, as it is biologically plausible that antimicrobial responses *in vitro* and in patients are influenced by porin mutations in conjunction with other resistance mechanisms.

In conclusion, this pilot study indicates that carbapenem-colistin regimens are viable options against a subset of CR-Kp infections, but overall outcomes remain suboptimal. Our data suggest that antimicrobial MICs and/or molecular markers of resistance, such as the presence of particular carbapenemases and porin mutations, may be useful for identifying strains most likely to respond to carbapenem-colistin combinations. If our findings are validated at other centers and in larger populations, MICs and genotypes of CR-Kp strains should be considered by clinicians as they make treatment decisions, along with the type of infection being treated, underlying diseases, and immune status. There are limited numbers of new antimicrobials in development with activity against carbapenem-resistant pathogens (15). Phenotypic assays and molecular markers that predict antimicrobial responsiveness among CR-Kp strains may allow clinicians to reserve new agents for cases in which they are most needed.

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REFERENCES

1. **Qureshi ZA, Paterson DL, Potoski BA, Kilayko MC, Sandovsky G, Sordillo E, Polsky B, Adams-Haduch JM, Doi Y.** 2012. Treatment outcome of bacteremia due to KPC-producing Klebsiella pneumoniae: superiority of combination antimicrobial regimens. Antimicrob Agents Chemother **56:**2108–2113. http://dx.doi.org/10.1128/AAC.06268-11.
2. **Clancy CJ, Chen L, Shields RK, Zhao Y, Cheng S, Chavda KD, Hao B, Hong JH, Doi Y, Kwak EJ, Silveira FP, Abdel-Massih R, Bogdanovich T, Humar A, Perlin DS, Kreiswirth BN, Hong Nguyen M.** 2013. Epidemiology and molecular characterization of bacteremia due to carbapenem-resistant Klebsiella pneumoniae in transplant recipients. Am J Transplant **13:**2619—2633. http://dx.doi.org/10.1111/ajt.12424.
3. **Tumbarello M, Viale P, Viscoli C, Trecarichi EM, Tumietto F, Marchese A, Spanu T, Ambretti S, Ginocchio F, Cristini F, Losito AR, Tedeschi S, Cauda R, Bassetti M.** 2012. Predictors of mortality in bloodstream infections caused by Klebsiella pneumoniae carbapenemase-producing K. pneumoniae: importance of combination therapy. Clin Infect Dis **55:**943–950. http://dx.doi.org/10.1093/cid/cis588.
4. **Jernigan MG, Press EG, Nguyen MH, Clancy CJ, Shields RK.** 2012. The combination of doripenem and colistin is bactericidal and synergistic against colistin-resistant, carbapenemase-producing Klebsiella pneumoniae. Antimicrob Agents Chemother **56:**3395–3398. http://dx.doi.org/10.1128/AAC.06364-11.
5. **Clancy CJ, Chen L, Hong JH, Cheng S, Hao B, Shields RK, Farrell AN, Doi Y, Zhao Y, Perlin DS, Kreiswirth BN, Nguyen MH.** 2013. Mutations of the ompK36 porin gene and promoter impact responses of sequence type 258, KPC-2-producing Klebsiella pneumoniae strains to doripenem and doripenem-colistin. Antimicrob Agents Chemother **57:**5258–5265. http://dx.doi.org/10.1128/AAC.01069-13.
6. **Daikos GL, Tsaousi S, Tzouvelekis LS, Anyfantis I, Psichogiou M, Argyropoulou A, Stefanou I, Sypsa V, Miriagou V, Nepka M, Georgiadou S, Markogiannakis A, Goukos D, Skoutelis A.** 2014. Carbapenemase-producing Klebsiella pneumoniae bloodstream infections: lowering mortality by antibiotic combination schemes and the role of carbapenems. Antimicrob Agents Chemother **58:**2322–2328. http://dx.doi.org/10.1128/AAC.02166-13.
7. **Tzouvelekis LS, Markogiannakis A, Piperaki E, Souli M, Daikos GL.** 2014. Treating infections caused by carbapenemase-producing Enterobacteriaceae. Clin Microbiol Infect **20:**862–872. http://dx.doi.org/10.1111/1469-0691.12697.
8. **Centers for Disease Control and Prevention.** 2013. Vital signs: carbapenem-resistant Enterobacteriaceae. MMWR Morb Mortal Wkly Rep **62:**165–170.
9. **Garonzik SM, Li J, Thamlikitkul V, Paterson DL, Shoham S, Jacob J, Silveira FP, Forrest A, Nation RL.** 2011. Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. Antimicrob Agents Chemother **55:**3284–3294. http://dx.doi.org/10.1128/AAC.01733-10.
10. **Clinical and Laboratory Standards Institute.** 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 8th ed. Approved standard M07-A8. Clinical and Laboratory Standards Institute, Wayne, PA.
11. **Fernandez-Cuenca F, Martinez-Martinez L, Conejo MC, Ayala JA, Perea EJ, Pascual A.** 2003. Relationship between beta-lactamase production, outer membrane protein and penicillin-binding protein profiles on the activity of carbapenems against clinical isolates of Acinetobacter baumannii. J Antimicrob Chemother **51:**565–574. http://dx.doi.org/10.1093/jac/dkg097.
12. **Hong JH, Clancy CJ, Cheng S, Shields RK, Chen L, Doi Y, Zhao Y, Perlin DS, Kreiswirth BN, Nguyen MH.** 2013. Characterization of porin expression in Klebsiella pneumoniae carbapenemase (KPC)-producing K. pneumoniae identifies isolates most susceptible to the combination of colistin and carbapenems. Antimicrob Agents Chemother **57:**2147–2153. http://dx.doi.org/10.1128/AAC.02411-12.
13. **Ho VP, Jenkins SG, Afaneh CI, Turbendian HK, Nicolau DP, Barie PS.** 2011. Use of meropenem by continuous infusion to treat a patient with a Bla(kpc-2)-positive Klebsiella pneumoniae blood stream infection. Surg Infect (Larchmt) **12:**325–327. http://dx.doi.org/10.1089/sur.2010.072.
14. **Van Wart SA, Andes DR, Ambrose PG, Bhavnani SM.** 2009. Pharmacokinetic-pharmacodynamic modeling to support doripenem dose regimen optimization for critically ill patients. Diagn Microbiol Infect Dis **63:**409–414. http://dx.doi.org/10.1016/j.diagmicrobio.2009.01.027.
15. **Shlaes DM.** 2013. New beta-lactam-beta-lactamase inhibitor combinations in clinical development. Ann N Y Acad Sci **1277:**105–114. http://dx.doi.org/10.1111/nyas.12010.