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SUSCEPTIBILITY

Clonal Background, Resistance Gene Profile, and Porin Gene Mutations Modulate *In Vitro* Susceptibility to Imipenem-Relebactam in Diverse *Enterobacteriaceae*

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ABSTRACT Treatment options for carbapenem-resistant *Enterobacteriaceae* (CRE) are limited. While *Klebsiella pneumoniae* strains harboring bla_{KPC} account for most CRE, recent evidence points to increasing diversification of CRE. We determined whether the CRE species and antibiotic resistance genotype influence the response to relebactam (REL), a novel beta-lactamase inhibitor with class A/C activity, combined with imipenem-cilastatin (IMI). We carried out broth microdilution testing with IMI alone or in the presence of 4 μg/ml REL against 154 clinical isolates collected at a New York City hospital with a high prevalence of organisms carrying bla_{KPC}, including *Enterobacter* spp. ($n = 96$), *K. pneumoniae* ($n = 44$), *Escherichia coli* ($n = 1$), *Serratia marcescens* ($n = 9$), and *Citrobacter* spp. ($n = 4$). Resistance gene profiles and the presence of major porin gene disruptions were ascertained by whole-genome sequencing. Addition of REL decreased the IMI MIC to the susceptible range ($\leq$1 μg/ml) against 88% of isolates. However, *S. marcescens* IMI-REL MICs were 4- to 8-fold higher than those for other organisms. Most bla_{KPC}-positive isolates had IMI-REL MICs of $\leq$1 μg/ml (88%), including isolates of *Enterobacter cloacae* ST171 (93%) and *K. pneumoniae* ST258 (82%). Nineteen isolates had IMI-REL MICs of $\geq$2 μg/ml, among which 84% harbored bla_{KPC} and one was bla_{NDM-1} positive. Isolates with IMI-REL MICs of $\geq$2 μg/ml versus those with MICs of $\leq$1 μg/ml were significantly more likely to demonstrate disruption of at least one porin gene (42% versus 19%; $P = 0.04$), although most *S. marcescens* isolates (67%) had intact porin genes. In conclusion, while REL reduced IMI MICs in a majority of diverse CRE isolates, including high-risk clones, chromosomal factors had an impact on IMI-REL susceptibilities and may contribute to elevated MICs for *S. marcescens*.

KEYWORDS *Enterobacteriaceae*, carbapenem resistance, imipenem-relebactam

The global dissemination of carbapenem-resistant *Enterobacteriaceae* (CRE) as a result of plasmid-mediated carbapenemases is a major threat to health care (1, 2). In addition to their resistance to beta-lactams, considered the mainstay of therapy against *Enterobacteriaceae*, CRE are typically resistant to multiple additional antimicrobial classes, which severely restricts treatment options. Moreover, antibiotics currently used to treat CRE infections, such as polymyxins, aminoglycosides, and tigecycline, are associated with high toxicity and relatively low efficacy (3). Among novel treatment approaches, beta-lactamase inhibitors with broad-spectrum activity are promising due to their ability to diminish cephalosporin or carbapenem MICs to the susceptible range, potentially restoring the activity of these potent drugs. Relebactam (REL) is a diazobicyclo-octane beta-lactamase inhibitor with class A/C activity (4) that is currently in phase 3 clinical development in combination with imipenem-cilastatin (IMI). The

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TABLE 1 Species-specific isolate susceptibilities to imipenem and imipenem-relebactam[a]

Species or ST	*n*	MIC_{50} (μg/ml)		MIC_{90} (μg/ml)		MIC range (μg/ml)		% of isolates with MIC of ≤1 μg/ml	
		IMI	IMI-REL	IMI	IMI-REL	IMI	IMI-REL	IMI	IMI-REL
All	154	8	0.5	64	2	1–≥128	≤0.125–8	<1	88
K. pneumoniae	44	8	0.5	64	1	4–≥128	≤0.125–8	0	84
ST258	28	16	0.5	128	2	4–≥128	0.125–8	0	82
Other STs[b]	16	8	0.5	≥128	8	4–≥128	0.25–8	0	88
Enterobacter spp.	96	8	0.25	64	1	1–≥128	≤0.125–8	1	92
E. cloacae	94	8	0.25	32	2	1–≥128	≤0.125–8	1	91
E. aerogenes	2	NA	NA	NA	NA	8	1–8	0	50
ST171	56	16	0.25	64	1	4–≥128	≤0.125–4	0	93
ST78	8	8	0.25	128	0.5	4–128	0.25–0.5	0	100
Other STs[c]	30	8	0.25	16	2	1–32	0.125–8	3	93
Serratia marcescens	9	16	1	32	8	4–≥128	0.25–8	0	67
Citrobacter spp.	4	4	0.25	8	2	4–8	≤0.125–2	0	75
C. freundii	2	NA	NA	NA	NA	4	≤0.125–0.25	0	100
C. koseri	1	NA	NA	NA	NA	8	0.5	0	100
C. braakii	1	NA	NA	NA	NA	8	2	0	0
E. coli	1	NA	NA	NA	NA	8	0.25	0	100

[a]Abbreviations: IMI, imipenem; REL, relebactam; ST, sequence type; NA, not applicable.
[b]ST17 ($n = 3$), ST307 ($n = 3$), ST392 ($n = 3$), and singletons ($n = 7$).
[c]ST62 ($n = 2$), ST133 ($n = 2$), ST137 ($n = 3$), and singletons ($n = 23$); does not include *E. aerogenes*.

spectrum of REL inhibition includes the *Klebsiella pneumoniae* carbapenemase (KPC), a class A carbapenemase widely distributed throughout the United States and worldwide (5). However, few previous studies have systematically examined IMI-REL activity against a diverse clinical collection of CRE strains harboring various beta-lactamase genes, including bla_{KPC}.

Several recent reports evaluating the activity of IMI-REL against Gram-negative bacteria demonstrated IMI MICs decreased to the susceptible range (≤1 μg/ml) for 67 to 100% of IMI-nonsusceptible *Enterobacteriaceae* following addition of REL (6–8). However, these studies included a small proportion of bla_{KPC}-producing isolates, the majority of which were *K. pneumoniae*, and molecular data were not provided to distinguish between isolate genotypes and bla_{KPC} subtypes. A recent study using whole-genome sequencing (WGS) to determine mechanisms of resistance to ceftazidime combined with avibactam, another broad-spectrum beta-lactamase inhibitor, and IMI-REL limited the molecular analysis to *K. pneumoniae* isolates (9). As the use of molecular diagnostics becomes increasingly available in clinical settings, it will be critical to identify molecular markers predicting antimicrobial efficacy in order to guide therapeutic decision-making. In the present study, we assessed eight different species of CRE to determine if the CRE species, clonal lineage, and resistance gene profile, including the bla_{KPC} subtype, influence the response to IMI-REL.

RESULTS

Susceptibilities across all isolates. We identified 154 CRE isolates collected from 117 patients, including 96 *Enterobacter*, 44 *K. pneumoniae*, 9 *Serratia marcescens*, 4 *Citrobacter*, and 1 *Escherichia coli* isolate, for further susceptibility testing (see Table S1 in the supplemental material). The most common culture site was the respiratory tract (37%), followed by blood (34%) and the urinary tract (14%). A small proportion of isolates (5%) were acquired from rectal surveillance swabs (Table S1). Among patients with multiple isolates collected, 6/18 (33%) harbored multiple species or different sequence types (STs). The IMI MIC_{50} and MIC_{90} values for all CRE isolates were 8 μg/ml and 64 μg/ml, respectively (Table 1). With the addition of REL, the IMI MIC_{50} and MIC_{90} values were reduced to 0.5 μg/ml and 2 μg/ml, respectively, and 135 (87%) isolates

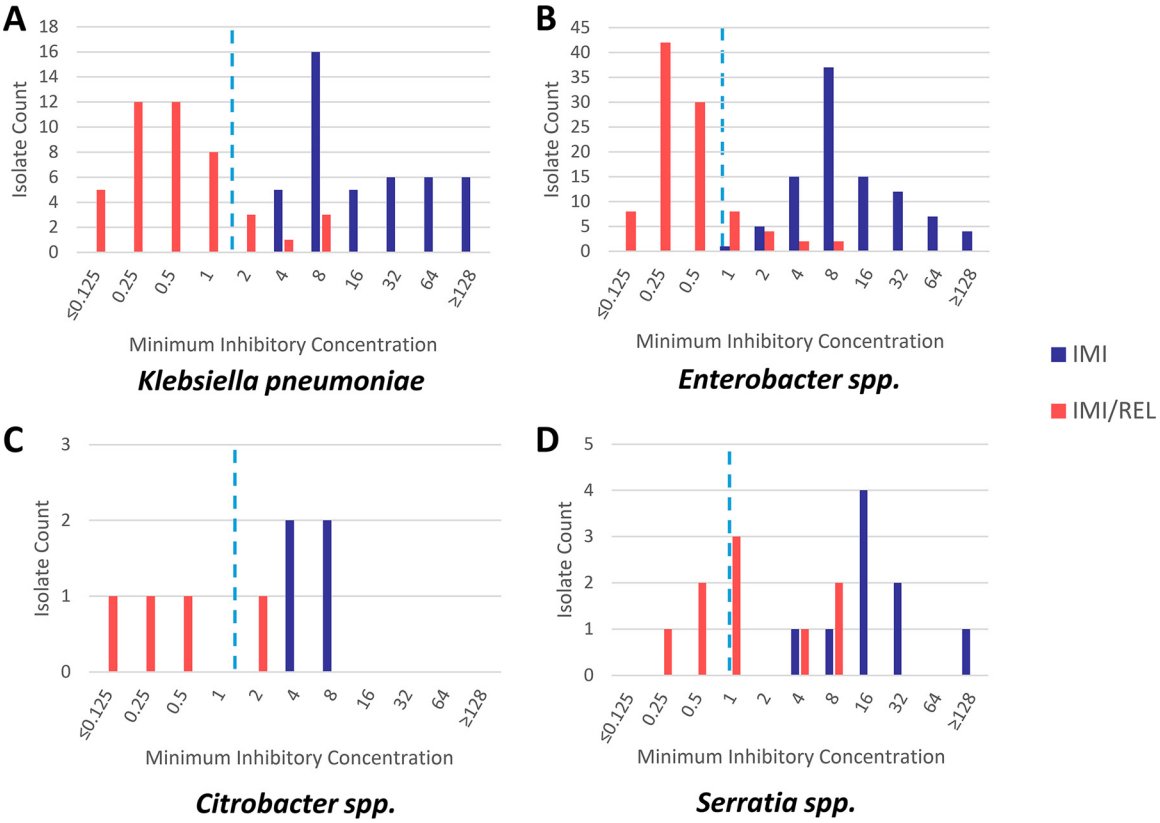

FIG 1 Distributions of IMI and IMI-REL MICs for various species of *Enterobacteriaceae*. The numbers of isolates with the indicated MICs of IMI and IMI-REL are shown for *Klebsiella pneumoniae* (A), *Enterobacter* spp. (B), *Citrobacter* spp. (C), and *Serratia* spp. (D). Most isolates of *Klebsiella pneumoniae* and *Enterobacter* spp., the most common carbapenem-resistant *Enterobacteriaceae*, demonstrated similar MIC distributions for IMI and IMI-REL, including reduction of IMI MICs to the susceptible range with addition of REL for the majority of isolates. Conversely, *Citrobacter* spp. demonstrated lower IMI MICs, while *Serratia* sp. MICs were higher overall, including a larger proportion of *Serratia* isolates with IMI-REL MICs of ≥2 μg/ml.

demonstrated IMI MICs in the susceptible range. This corresponded to a median reduction in IMI MIC of 32-fold (interquartile range [IQR], 16- to 64-fold) across all isolates. Overall, 19 (12%) isolates were found to have IMI-REL MICs of ≥2 μg/ml. Among 68 (44%) isolates with high-level IMI resistance (MIC ≥ 16 μg/ml), the IMI MIC_{50} and MIC_{90} values were reduced from 32 and 128 μg/ml to 0.5 and 4 μg/ml, respectively, with the addition of REL. This corresponded to a decrease in IMI MIC to the susceptible range in 56/68 (82%) isolates with high-level IMI resistance, compared to 79/86 (92%) isolates with IMI MICs of <16 μg/ml. No isolates had an IMI-REL MIC of >8 μg/ml.

Species-specific susceptibilities. Species-specific IMI and IMI-REL MIC values are summarized in Table 1. All tested species of *Enterobacteriaceae* demonstrated comparable IMI MIC_{50} and MIC_{90} values (MIC_{50} and MIC_{90} ranges of 4 to 16 and 8 to 64 μg/ml, respectively), with the exception of *Citrobacter* spp., which had MICs 2- to 8-fold lower than those for other organisms (Fig. 1). Likewise, IMI-REL MIC_{50} and MIC_{90} values were similar among *K. pneumoniae*, *Enterobacter*, and *Citrobacter* isolates (MIC_{50} and MIC_{90} ranges of 0.25 to 0.5 and 0.25 to 2 μg/ml, respectively). However, *S. marcescens* demonstrated IMI-REL MICs that were 4- to 8-fold higher than those for other organisms (MIC_{50} and MIC_{90} of 1 and 8 μg/ml, respectively) (Fig. 1). Among *Enterobacter* isolates, REL increased the percentage of isolates with IMI MICs of ≤1 μg/ml from 1 to 92% (Table 1), corresponding to a median change in IMI MIC of 32-fold. Addition of REL also reduced IMI MICs to ≤1 μg/ml for 84% of *K. pneumoniae* isolates, 75% of *Citrobacter* isolates, and the *E. coli* isolate (median changes of 32-, 16-, and 32-fold, respectively). Addition of REL reduced IMI MICs to the susceptible range in a smaller proportion (67%) of *S. marcescens* isolates than that of other organisms (89%), corre-

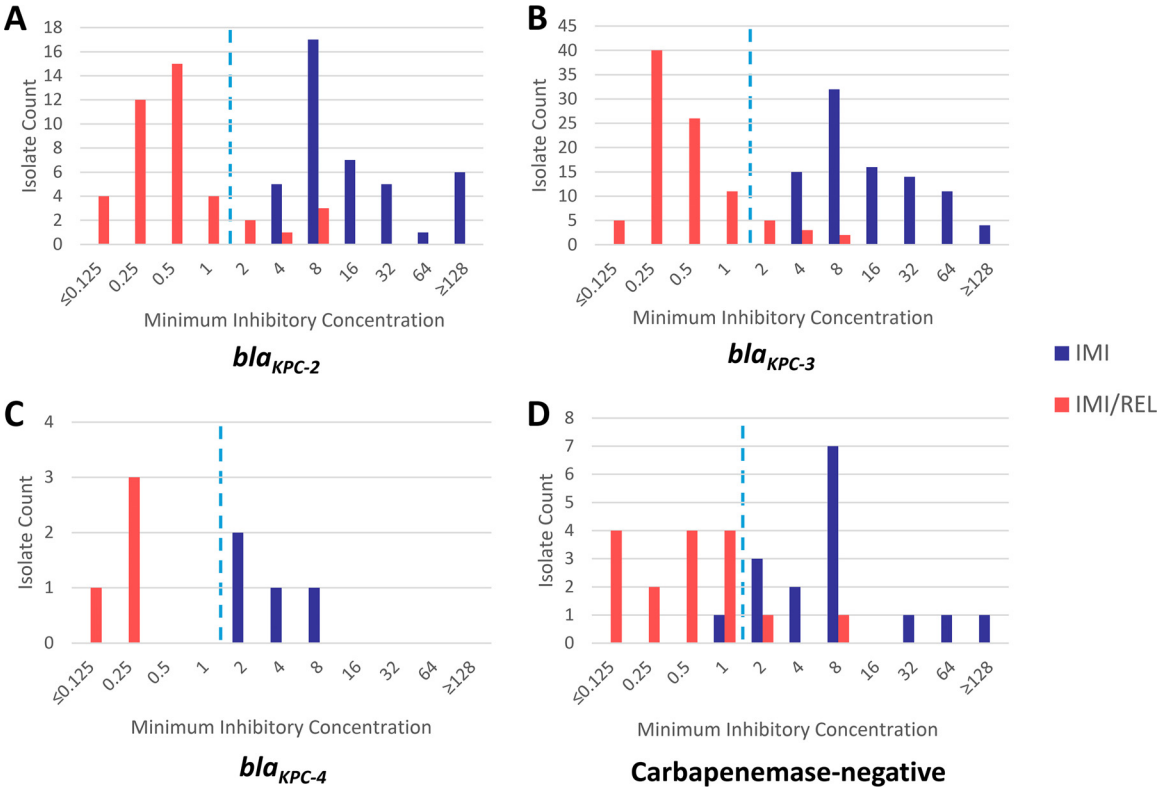

FIG 2 IMI and IMI-REL MICs for carbapenem-resistant *Enterobacteriaceae* (CRE) according to bla_{KPC} carriage. The IMI and IMI-REL MICs were determined for isolates that harbored three different subtypes of bla_{KPC}, i.e., bla_{KPC-2} (A), bla_{KPC-3} (B), and bla_{KPC-4} (C), or were carbapenemase negative (D). MICs for bla_{KPC-2}- and bla_{KPC-3}-positive isolates, which also constituted the majority of CRE isolates, were comparable, while isolates harboring bla_{KPC-4} demonstrated lower MICs for both IMI and IMI-REL. IMI and IMI-REL MICs for carbapenemase-negative isolates were highly variable, suggesting a range of different mechanisms of resistance in these isolates.

sponding to a median 10-fold reduction in IMI-REL MICs. The proportion of isolates with IMI-REL MICs of ≥2 μg/ml was significantly higher for *S. marcescens* than for *Enterobacter* spp. ($P = 0.03$) but not for other organisms.

We further characterized *Enterobacter cloacae* complex (which includes *Enterobacter asburiae*), *K. pneumoniae*, and *Citrobacter freundii* isolates by using multilocus sequence typing (MLST) (Table 1). The most commonly identified clones in this collection were *K. pneumoniae* ST258 ($n = 28$) and *E. cloacae* ST171 ($n = 56$) and ST78 ($n = 8$). No other STs included more than four isolates. For ST258 and ST171, similar proportions of isolates were susceptible to IMI-REL compared to those for other *K. pneumoniae* and *E. cloacae* isolates (82% and 93%, respectively), while ST78 isolates were uniformly susceptible to IMI-REL (Table 1).

Resistance mechanisms. Among 137 isolates harboring bla_{KPC}, including 82 *Enterobacter*, 42 *K. pneumoniae*, 8 *S. marcescens*, 4 *Citrobacter*, and 1 *E. coli* isolate, addition of REL reduced IMI MICs to ≤1 μg/ml for 88% of isolates. *Enterobacter* spp. (14%) included the largest proportion of bla_{KPC}-negative isolates, while only 5% of *K. pneumoniae* isolates lacked bla_{KPC}. IMI and IMI-REL MICs were more variable for bla_{KPC}-negative isolates ($n = 17$) (Fig. 2). However, the proportions of bla_{KPC}-positive and -negative isolates demonstrating IMI-REL MICs of ≤1 μg/ml did not significantly differ (88% versus 82%; $P = 0.4$). Our collection included one isolate with a non-bla_{KPC} carbapenemase gene, bla_{NDM-1} (*E. cloacae*), which was resistant to both IMI and IMI-REL (MICs of 16 and 8 μg/ml, respectively), consistent with the known lack of activity of REL against class B and D carbapenemases (10).

For isolates harboring bla_{KPC-2} ($n = 41$) and bla_{KPC-3} ($n = 92$), the most commonly encountered bla_{KPC} subtypes (30% and 67% of bla_{KPC}-positive isolates, respectively), IMI and IMI-REL MIC_{50} and MIC_{90} values were similar (IMI MIC_{50} of 8 μg/ml for both

TABLE 2 Effects of resistance gene subtypes on imipenem and imipenem-relebactam susceptibilities[a]

Resistance mechanism	*n*	MIC_{50} (μg/ml)		MIC_{90} (μg/ml)		MIC range (μg/ml)		% of isolates with MIC of <1 μg/ml	
		IMI	IMI-REL	IMI	IMI-REL	IMI	IMI-REL	IMI	IMI-REL
bla_{KPC}	137	8	0.5	64	2	2–≥128	0.125–8	0	88
bla_{KPC-2}	41	8	0.5	128	2	4–≥128	0.125–8	0	85
bla_{KPC-3}	92	8	0.5	64	2	4–≥128	0.125–8	0	89
bla_{KPC-4}	4	2	0.25	8	0.25	2–8	0.125–0.25	0	100
bla_{NDM-1}	1	NA	NA	NA	NA	16	8	0	0
Carbapenemase negative	16	8	0.5	64	2	1–128	≤0.125–8	6	88

[a]Abbreviations: IMI, imipenem; REL, relebactam; NA, not available.

subtypes; IMI MIC_{90}s of 128 and 64 μg/ml, respectively; and IMI-REL MIC_{50} of 0.5 μg/ml and IMI-REL MIC_{90} of 2 μg/ml for both subtypes) (Table 2). Among isolates harboring bla_{KPC-2}, 85% had IMI-REL MICs of ≤1 μg/ml, compared to 89% of bla_{KPC-3}-positive isolates. However, isolates harboring bla_{KPC-4} ($n = 4$) demonstrated 2- to 8-fold lower IMI and IMI-REL MIC_{50} and MIC_{90} values than those for isolates harboring bla_{KPC-2} or bla_{KPC-3}; 100% of these isolates had IMI MICs of ≤1 μg/ml following addition of REL (Table 2). Differences across bla_{KPC} subtypes did not meet the criteria for statistical significance.

Overall, the CRE isolates included in this study carried a remarkably large number of genes encoding resistance to multiple drug classes, including beta-lactams, aminoglycosides, fluoroquinolones, and tetracyclines. The median number of resistance genes detected was 11 (range, 1 to 20; IQR, 10 to 14). In addition to bla_{KPC}, the most commonly detected beta-lactamase gene families included bla_{TEM} (79% of isolates), bla_{OXA} (73% of isolates), and bla_{AmpC} (70% of isolates); 72% of isolates harbored ≥4 beta-lactamase genes. Compared to isolates harboring bla_{KPC} or other carbapenemase genes, carbapenemase-negative isolates harbored significantly fewer beta-lactamase genes (median of 4 [IQR, 4 to 5] versus median of 1 [IQR, 1 to 2]; $P < 0.0001$). However, all carbapenemase-negative isolates carried either bla_{AmpC} (14/16 isolates) or $bla_{CTX-M-15}$ (2/16 isolates). The numbers of total beta-lactamases in isolates with IMI-REL MICs of ≤1 μg/ml and those with IMI-REL MICs of ≥2 μg/ml were similar (median of 4 [IQR, 3 to 5] for both categories; $P = 0.5$). No individual beta-lactamase family was overrepresented among IMI-REL-resistant isolates. Among carbapenemase-negative isolates that were nonsusceptible to IMI-REL ($n = 2$), both were *E. cloacae* isolates which harbored only bla_{AmpC}.

Comparison of porin gene sequences to wild-type references revealed evidence of major disruptions likely leading to loss of function of the *ompC/ompK36* porin gene in 27 (18%) isolates and of the *ompF/ompK35* gene in 19 (12%) isolates. Among carbapenemase-negative isolates, 9/16 (56%) had a major disruption in at least one porin gene, compared to 25/138 (18%) carbapenemase-positive isolates ($P = 0.002$). Isolates with IMI-REL MICs of ≥2 μg/ml were also significantly more likely than isolates with IMI-REL MICs of ≤1 μg/ml to have a putative loss-of-function mutation in at least one of the two genes (8/19 isolates [42%] versus 26/135 isolates [19%]; $P = 0.04$). Although significant differences in individual genes were not seen, a larger proportion of isolates with IMI-REL MICs of ≥2 μg/ml harbored major disruptions in *ompC/ompK36* than the proportion harboring major disruptions in *ompF/ompK35* (6/19 isolates [22%] versus 2/19 isolates [11%]). This included two carbapenemase-negative *E. cloacae* isolates with IMI-REL MICs of ≥2 μg/ml, of which one had multiple small deletions causing a frameshift and the other demonstrated a deletion leading to a premature stop codon in *ompC*. Putative loss-of-function porin gene mutations may also have contributed to elevated IMI-REL MICs in 4/7 (57%) isolates with an elevated IMI-REL MIC of 8 μg/ml. However, only 3/9 (33%) *S. marcescens* isolates harbored major porin gene disruptions, suggesting an alternative intrinsic mechanism for the poor response to REL in this species.

DISCUSSION

Addition of beta-lactamase inhibitors to carbapenem therapy represents a promising strategy for restoring efficacy of these highly potent antibiotics. While CRE represent key targets for novel antibiotic development, the increasing diversity of these pathogens indicates a need to examine drug efficacy in bacteria from a range of species and with a range of carbapenem resistance mechanisms. In this study, we found that addition of REL decreased IMI MICs to the susceptible range for 88% of clinical CRE isolates from an area of high bla_{KPC} endemicity. Although several previous studies reported reductions in IMI MICs for a larger proportion of multidrug-resistant *Enterobacteriaceae*, these studies included few CRE isolates (5–8). In a recent study of CRE isolates, addition of REL similarly increased the proportion of IMI-susceptible isolates, from 8% to 88%, with most of the susceptible isolates being *K. pneumoniae* (9). Here we found that for *K. pneumoniae*, *E. cloacae*, and *Citrobacter* spp., REL reduced IMI MICs to $\leq$1 μg/ml in a large proportion of isolates. However, REL was less effective at decreasing IMI MICs in a small collection of *S. marcescens* isolates. Isolates expressing three different subtypes of bla_{KPC} also largely had IMI-REL MICs of $\leq$1 μg/ml, although carbapenemase-negative samples demonstrated variable IMI-REL MICs.

Our study includes a large collection of carbapenem-resistant *Enterobacter* isolates, which reflects the rising burden of carbapenem-resistant *E. cloacae* (CREC) in the United States (11–15). CREC isolates largely demonstrated IMI-REL MICs of $\leq$1 μg/ml, including isolates belonging to two widely reported CREC clones, ST171 (93%) and ST78 (100%). REL also reduced IMI MICs to the susceptible range for >80% of carbapenem-resistant *K. pneumoniae* (CRKP) isolates, including the epidemic clone ST258 (82%). In addition to broad-spectrum beta-lactam resistance, CREC and CRKP are known to demonstrate remarkable cross-class antibiotic resistance, which severely limits treatment options (16). In this analysis, these isolates harbored as many as 20 antibiotic resistance genes, conferring resistance to multiple antibiotic classes, including aminoglycosides, fluoroquinolones, sulfonamides, and tetracyclines. Taken together, these data suggest that IMI-REL may have an important role in treating infections caused by these two CRE species, including outbreak strains.

Among all tested isolates, IMI-REL demonstrated the lowest efficacy against *S. marcescens*, which had IMI-REL MIC_{50} and MIC_{90} values 4 to 8 times higher than those of other species. This is of particular concern because *Serratia* spp. are intrinsically resistant to polymyxins, and as such, novel carbapenemase inhibitors may provide much-needed alternative treatment options. Because *Serratia* spp., including *S. marcescens*, lack MLST or other reproducible genotyping schemes, isolate diversity could not readily be assessed in this study. Potential mechanisms of resistance may include changes in cell membrane permeability, perhaps attributable to porin gene mutations (17, 18), although we found evidence for significant porin gene disruptions in less than half of these *Serratia* isolates, suggesting an alternative, species-specific mechanism of resistance. Notably, all *S. marcescens* isolates were also found to harbor bla_{AmpC} by reference mapping after an automated resistance gene detection pipeline failed to identify this gene. This was most likely due to allelic heterogeneity of bla_{AmpC} in *Serratia* spp. (19) and may reflect relatively high diversity within this species.

While bla_{KPC} accounts for the majority of CRE strains in the United States, a variety of resistance mechanisms have been found to contribute to elevated carbapenem MICs. bla_{KPC-2} and bla_{KPC-3} are the most common bla_{KPC} subtypes, while other bla_{KPC} subtypes and carbapenemases remain relatively uncommon (2). For bla_{KPC-2}- and bla_{KPC-3}-harboring isolates, REL resulted in large reductions in IMI MICs, suggesting that IMI-REL may be an important option for treating these bacteria alone or as part of combination regimens, where carbapenem MICs may have substantial impacts on treatment responses (20, 21). Notably, bla_{KPC-4}-positive isolates demonstrated particularly low IMI-REL MICs, raising the possibility that either the bla_{KPC-4} allele harbors mutations that alter beta-lactamase activity or the coexpression of chromosomal genes modulates IMI-REL MICs in these isolates. While resistance to IMI-REL was expected in bla_{NDM-1}-positive isolates, based

on previous studies (5), we have not found evidence of spread of non-bla_{KPC}-encoded carbapenemases at our hospital. In carbapenemase-negative isolates, the combination of AmpC overproduction with porin gene mutations results in carbapenem resistance in a wide range of isolates (18, 22). In two carbapenemase-negative isolates in this collection with IMI-REL MICs of $\geq$2 μg/ml, we detected both bla_{AmpC} and a porin gene disruption, suggesting that cumulative changes in the core chromosome may have a significant impact on IMI-REL MICs. Consistent with our findings, in a recent analysis of factors associated with IMI-REL MICs in bla_{KPC}-positive *K. pneumoniae*, major *ompK36* mutations, but not the bla_{KPC} subtype or ESBL gene carriage, were independently associated with increased MICs (9).

Another beta-lactam–beta-lactamase inhibitor combination approved for clinical use in 2015, ceftazidime-avibactam, also has activity against ESBL-producing *Enterobacteriaceae* and many carbapenemase producers, including bla_{KPC}- and bla_{OXA-48}-positive but not other metallo-beta-lactamase-producing isolates (23). Ceftazidime-avibactam has received FDA approval for use in patients with hospital-acquired pneumonia, including ventilator-associated infections (24) and complicated intra-abdominal (25–27) and urinary tract (26, 28, 29) infections, based on several phase III trials, although these studies included few CRE isolates. Moreover, a recent study comparing clinical outcomes for patients with carbapenemase-producing bacteria treated with ceftazidime-avibactam compared to those treated with colistin showed an all-cause in-hospital 30-day mortality of 3% for patients who received ceftazidime-avibactam first, compared to 33% for patients who received colistin first (30). However, development of resistance during therapy as a result of mutations in bla_{KPC}, bla_{KPC} duplications, and/or chromosomal modifications presents a challenge to the clinical use of ceftazidime-avibactam, particularly in patients with severe infections (31, 32). Compared to those with avibactam, REL appeared to form more stable acyl complexes with KPC-2, leading to more effective inhibition, although the clinical implications of this are unclear (21). As IMI-REL enters clinical use for patients with similar indications, further assessments are needed to determine whether resistance to IMI-REL may similarly develop during treatment.

While this study was conducted at a single hospital center, our results are strengthened by our diverse isolate collection, which was enriched by a large number of CREC isolates as well as a unique selection of carbapenem-resistant *S. marcescens* and *Citrobacter* isolates. The large proportion of isolates harboring bla_{KPC} reflects the major contribution of this carbapenemase to CRE in the United States, while other carbapenemases were uncommon. We were able to use a comprehensive screening tool for antibiotic resistance genes, which enabled detection of carbapenemase and other beta-lactamase genes, although we also encountered limitations in detection of bla_{AmpC}, a potential contributor to carbapenem resistance in these isolates. We also were able to derive genotyping data for the majority of isolates in this collection, including assessment of porin genes for mutations likely to lead to loss of function. However, assessment of the relatedness of *S. marcescens*, non-*freundii Citrobacter*, and *Enterobacter aerogenes* isolates was beyond the scope of this study, as a rapid genotyping scheme is unavailable for these organisms. Because this study tested IMI-REL MICs *in vitro* and MIC breakpoints are not yet available for IMI-REL, we based our assessment on the reduction of IMI MICs to the susceptible range following addition of REL. Clinical studies of IMI-REL efficacy may lead to establishment of different MIC breakpoints. Functional studies are also needed to better define porin gene disruptions and their impact on IMI-REL MICs.

In summary, we found most isolates in a large, diverse collection of clinical CRE isolates to have IMI MICs in the susceptible range following addition of REL, suggesting that this is a promising option for restoring carbapenem efficacy in these Gram-negative bacteria. Importantly, REL reduced IMI MICs to $\leq$1 μg/ml in a large proportion of bla_{KPC}-harboring CRE isolates. However, we also detected elevated IMI-REL MICs for *S. marcescens* and variable MICs for carbapenemase-negative isolates, supporting the presence of species-specific resistance patterns and the potential for chromosomally

encoded mechanisms to modulate MICs. Additional studies are needed to identify the basis of increased MICs for *S. marcescens* and other isolates demonstrating elevated IMI-REL MICs. Taken together, our findings suggest that rapid identification of bacterial species and carbapenemase carriage may enable early, empirical use of IMI-REL in appropriate clinical settings.

MATERIALS AND METHODS

Isolate collection. We obtained clinical CRE isolates collected between 2010 and 2016 from the clinical microbiology laboratory at our tertiary care hospital in New York City, which routinely stores multidrug-resistant isolates for quality control and research purposes. Antimicrobial susceptibilities were determined using Vitek2 or Etest (bioMérieux) assay as part of routine medical diagnostics and were interpreted using recent Clinical and Laboratory Standards Institute (CLSI) MIC breakpoints (33). Isolates nonsusceptible to IMI (MIC $\geq$ 2 μg/ml) or meropenem (MER) (MIC $\geq$ 2 μg/ml) were selected for additional microbiological and genomic analyses. This included a random selection of approximately every 5th available *K. pneumoniae* isolate, predominantly representing bloodstream isolates. We also included all available non-*K. pneumoniae* CRE isolates. The culture source was ascertained from patient clinical records. Study procedures were approved by the Columbia University Medical Center Institutional Review Board.

Microbiological analysis. We carried out broth microdilution testing with IMI alone or in the presence of 4 μg/ml REL (Merck & Co., Inc.) in order to quantify antimicrobial activity against CRE isolates. IMI and IMI in combination with REL were dissolved in a phosphate buffer solution, and broth microdilution was performed in accordance with the CLSI reference method. Twofold dilutions of IMI or IMI-REL, corresponding to MIC ranges of 0.5 to 128 μg/ml for IMI and 0.0625 to 16 μg/ml for IMI-REL, were added to 2-ml bacterial inocula in untreated 96-well plates, mixed, and incubated overnight at 37°C. The MIC_{50} and MIC_{90} were defined as the antimicrobial concentrations that inhibited growth of 50% and 90% of bacterial isolates, respectively. Given that IMI-REL MIC breakpoints have not yet been defined, we assessed the reduction of IMI MICs to the susceptible range ($\leq$1 μg/ml) following addition of REL.

Isolate sequencing, genotyping, and porin gene analysis. Mechanisms of resistance, including KPC subtypes, were ascertained by WGS. Genomic DNA was extracted using a DNeasy UltraClean microbial kit (Qiagen) following the manufacturer's instructions. Unique index-tagged libraries consisting of 125- to 300-bp paired-end reads were generated for each isolate for WGS, which was performed using a HiSeq 2500 sequencer (Illumina) at the New York Genome Center or a MiSeq sequencer (Illumina) at the Columbia Microbiome & Pathogen Genomics Core (see Table S1 in the supplemental material). A subset of *E. cloacae* isolates was sequenced for a previous analysis (NCBI Sequencing Read Archive [SRA] accession numbers SRP099597 and SRP126514) (Table S1) (34). We then used SRST2 (35) to perform isolate characterization from resulting Illumina reads, including derivation of the isolate MLST (36–39), if available for the given species, and antimicrobial resistance gene profiling using the ARG-ANNOT database (40). To identify bla_{AmpC} carriage in *S. marcescens*, Illumina sequencing reads were aligned against a reference sequence from an SRT/SST family class C beta-lactamase (NCBI GenBank accession number WP_063842944.1) and further analyzed using BLAST. To assess porin gene sequences, Illumina reads were mapped against publicly available, wild-type *ompC/ompK36* and *ompF/ompK35* porin gene reference sequences by use of Geneious v10.2.4 (Biomatters Ltd.). Wild-type reference sequences were derived from the following reference isolates: *E. cloacae* ATCC 13047 (NCBI GenBank accession numbers YP_003614002.1 and YP_003613214.1), *E. aerogenes* ATCC 13048 (accession numbers AAK11270.1 and AAR84609.1), *K. pneumoniae* ATCC 13883 (accession numbers KFJ75664.1 and KFJ72617.1), *E. coli* K-12 (accession numbers NP_416719.1 and NP_415449.1), *C. freundii* ATCC 8090 (accession numbers EKS56410.1 and EKS58001.1), and *S. marcescens* ATCC 13880 (accession numbers KFD13790.1 and KFD11799.1). Putative loss-of-function mutations were defined as those resulting in premature stop codons, frameshift mutations, or large deletions with potential functional ramifications. Mutations distributed across a clonal lineage without clear functional implications were excluded.

Statistical methods. Isolate groups were compared using the chi-square or Fisher exact test for categorical variables and the Mann-Whitney U or Kruskal-Wallis test for continuous variables, as appropriate. For all analyses, *P* values of $<$0.05 were considered statistically significant. Statistical analyses were conducted in SAS v9.4 (SAS Institute Inc.).

Accession number(s). The sequences obtained in this study were deposited in the NCBI Sequencing Read Archive (SRA) under accession number SRP135817.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00573-18.

SUPPLEMENTAL FILE 1, XLSX file, 0.1 MB.

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We have no additional conflicts of interest to disclose.

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