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Activity of Imipenem-Relebactam and Comparator Agents against Genetically Characterized Isolates of Carbapenem-Resistant *Enterobacteriaceae*

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ABSTRACT Carbapenem-resistant *Enterobacteriaceae* (CRE) strains are an urgent public health threat. We evaluated the *in vitro* activities of 19 antimicrobial agents, including imipenem-relebactam, against (i) 106 CRE bloodstream isolates that primarily expressed *Klebsiella pneumoniae* carbapenemase (KPC) and (ii) 20 OXA-48-like-expressing CRE isolates. Ninety-five percent of CRE bloodstream isolates were susceptible to imipenem-relebactam. In contrast to their comparable activities against KPC-producing CRE strains, ceftazidime-avibactam was more active *in vitro* against OXA-48-like CRE strains than was imipenem-relebactam (90% susceptible versus 15% susceptible).

KEYWORDS carbapenem-resistant *Enterobacteriaceae*, ceftazidime-avibactam, imipenem-relebactam

Carbapenem-resistant *Enterobacteriaceae* (CRE) strains are an urgent public health threat (1). The emergence of CRE is due to the rapid expansion of plasmid-encoded, carbapenem-hydrolyzing enzymes (carbapenemases). *Klebsiella pneumoniae* carbapenemase (KPC) is the most common carbapenem resistance mechanism among *Enterobacteriaceae* strains in the United States, South America, Israel, Greece, and Italy. Other carbapenemase enzymes predominate in other countries, including OXA-48-type enzymes that are prevalent in Spain, France, Turkey, North Africa, and the Middle East (2–4). Relebactam and avibactam are novel diazabicyclooctanes that effectively inhibit KPC enzymes and inhibit some OXA-48-type carbapenemases (5, 6). Relebactam has been combined with imipenem and avibactam has been combined with ceftazidime to represent novel β-lactam/β-lactamase inhibitor combinations, which have great potential for the treatment of infections due to many CRE strains.

In this study, we compare the *in vitro* activity of imipenem-relebactam, ceftazidime-avibactam, and other potentially useful antimicrobial agents against a collection of 106 contemporary CRE bloodstream isolates and 20 OXA-48-like isolates that have been genetically characterized. Antimicrobial susceptibility testing was performed by reference broth microdilution to assess the activities of 19 antimicrobial agents, including penicillins (piperacillin-tazobactam and amoxicillin-clavulanate), cephalosporins (ceftazidime, ceftazidime-avibactam, and cefepime), a monobactam (aztreonam), carbapenems (ertapenem, imipenem, imipenem-relebactam, meropenem, and doripenem), a fluoroquinolone (ciprofloxacin), aminoglycosides (gentamicin and amikacin), a tetracycline (minocycline), a glycylcycline (tigecycline), a folate synthesis inhibitor (trimethoprim-sulfamethoxazole), and polymyxins (colistin and polymyxin B) (7). Isolate MICs were

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interpreted as susceptible, intermediate, or resistant for each of the antimicrobial agents based on Clinical and Laboratory Standards Institute (CLSI) breakpoints, where applicable (8). Isolates for which MIC values were $\leq$2 μg/ml for tigecycline (the FDA susceptible breakpoint when testing *Enterobacteriaceae*), colistin, and polymyxin B were considered susceptible to those agents. The CLSI interpretive criteria for imipenem were applied to imipenem-relebactam. The percentages of isolates that tested susceptible, intermediate, or resistant, as well as the MIC_{50} and MIC_{90}, were calculated for each of the antimicrobial agents.

The 106 CRE bloodstream isolates represented six unique species, namely, *Klebsiella pneumoniae* ($n = 97$), *Enterobacter cloacae* ($n = 3$), *Escherichia coli* ($n = 3$), *Morganella morganii* ($n = 1$), *Pluralibacter* (formerly *Enterobacter*) *gergoviae* ($n = 1$), and *Klebsiella* (formerly *Enterobacter*) *aerogenes* ($n = 1$) (see Table S1 in the supplemental material) (9). Carbapenemases were identified in 93.4% of the isolates (99/106 isolates). The 7 isolates without carbapenemases included *K. pneumoniae* ($n = 4$), *P. gergoviae* ($n = 1$), and *E. coli* ($n = 2$) (Table S2). The number of β-lactamases present per isolate was 3 ± 0.5 β-lactamases (median $\pm$ standard deviation).

Of the 99 carbapenemase-producing CRE isolates, KPC-2 and KPC-3 were present in 47 and 51 isolates, respectively. The remaining carbapenemase-producing isolate was an OXA-48-producing *K. pneumoniae* strain. No isolates with multiple carbapenemases were identified. Ninety-two of the carbapenem-resistant *K. pneumoniae* isolates expressed KPC, of which 84% (77/92 isolates) were sequence type 258 (ST258). In addition, 37% of isolates (34/92 isolates) harbored an extended-spectrum β-lactamase (ESBL). The most common *wzi* (a gene that encodes an outer membrane protein that correlates with capsular type [10]) alleles were *wzi154* (43/92 isolates), *wzi29* (19/92 isolates), *wzi50* (8/92 isolates), and *wzi83* (7/92 isolates). Forty-five KPC-expressing *K. pneumoniae* isolates (49% [45/92 isolates]) had mutations in the gene that encodes OmpK35, an outer membrane porin that allows for passage of carbapenems, and 31 (34% [31/92 isolates]) had cooccurrences of *ompK35* and *ompK36* mutations. Of note, no isolate had an *ompK36* mutation without an *ompK35* mutation.

The OXA-48-like CRE isolate collection was composed of five unique species (Table S3), namely, *K. pneumoniae* ($n = 14$), *Klebsiella* (formerly *Enterobacter*) *aerogenes* ($n = 2$), *Escherichia coli* ($n = 2$), *Proteus mirabilis* ($n = 1$), and *Klebsiella ozaenae* ($n = 1$). Eighty percent of those isolates (16/20 isolates) coharbored ESBLs. OXA-48 ($n = 8$) and OXA-181 ($n = 8$) enzymes were the most common, followed by OXA-232 ($n = 3$) and OXA-163 ($n = 1$). Porin mutations were present in 86% of *K. pneumoniae* isolates (12/14 isolates), including *ompK35* mutations alone ($n = 9$), *ompK36* mutations alone ($n = 2$), and cooccurrence of *ompK35* and *ompK36* mutations ($n = 1$).

Of the 19 antimicrobial agents evaluated for the CRE bloodstream isolates, only 8/19 agents (imipenem-relebactam, ceftazidime-avibactam, amikacin, gentamicin, minocycline, tigecycline, colistin, and polymyxin B) from 5/9 antimicrobial classes demonstrated *in vitro* activity against >50% of the strains tested, and only 2 (ceftazidime-avibactam and imipenem-relebactam) exhibited activity against >90% (Table S4). The imipenem-relebactam MIC_{50} and MIC_{90} were $\leq$0.25/4 μg/ml and 0.5/4 μg/ml, respectively. Notably, all isolates for which the MIC exceeded the mode for imipenem-relebactam harbored genes for multiple mechanisms of β-lactam resistance.

Of the 19 antimicrobial agents evaluated for activity against the OXA-48-like CRE isolates, only 4/19 agents (ceftazidime-avibactam, tigecycline, colistin, and polymyxin B) from 3/9 antimicrobial classes were active against >50% of the isolates, and only 3 (ceftazidime-avibactam, colistin, and polymyxin B) were active against >90% (Table S5). The addition of avibactam restored *in vitro* susceptibility to ceftazidime in 14/16 OXA-48-like strains that were resistant to ceftazidime alone. In contrast, the addition of relebactam to imipenem did not restore *in vitro* susceptibility to imipenem in any of the 17 strains that were resistant to imipenem alone. Of note, the MIC_{50} and MIC_{90} of ceftazidime-avibactam were unchanged in the OXA-48-like CRE isolates, compared to the CRE bloodstream isolates.

All 92 KPC-expressing *K. pneumoniae* bloodstream isolates were susceptible to

TABLE 1 Imipenem-relebactam MIC values for KPC-expressing *K. pneumoniae* bloodstream isolates stratified by ESBL status, ST258 status, *wzi* allele, and mutations in *ompK35* and *ompK36* outer membrane porin genes[a]

Isolate type	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)
KPC-expressing *K. pneumoniae* (*n* = 92)	≤0.25/4	0.5/4	≤0.25/4 to 1/4
ESBL (*n* = 34)	≤0.25/4	0.5/4	≤0.25/4 to 1/4
ST258 (*n* = 77)	≤0.25/4	0.5/4	≤0.25/4 to 1/4
Non-ST258 (*n* = 15)	≤0.25/4	≤0.25/4	≤0.25/4 to ≤0.25/4
wzi29 (*n* = 19)	≤0.25/4	0.5/4	≤0.25/4 to 1/4
wzi50 (*n* = 8)	≤0.25/4	0.5/4	≤0.25/4 to 0.5/4
wzi83 (*n* = 7)	≤0.25/4	1/4	≤0.25/4 to 1/4
wzi154 (*n* = 43)	≤0.25/4	0.5/4	≤0.25/4 to 1/4
Wild-type *ompK35* and *ompK36* (*n* = 15)	≤0.25/4	≤0.25/4	≤0.25/4 to ≤0.25/4
ompK35 mutation only (*n* = 45)	≤0.25/4	0.5/4	≤0.25/4 to 1/4
ompK35 and *ompK36* mutations (*n* = 31)	≤0.25/4	1/4	≤0.25/4 to 1/4
KPC-2-expressing *K. pneumoniae* (*n* = 44)	≤0.25/4	0.5/4	≤0.25/4 to 1/4
ESBL (*n* = 19)	≤0.25/4	0.5/4	≤0.25/4 to 1/4
ST258 (*n* = 35)	≤0.25/4	0.5/4	≤0.25/4 to 1/4
Non-ST258 (*n* = 9)	≤0.25/4	≤0.25/4	≤0.25/4 to ≤0.25/4
wzi29 (*n* = 18)	≤0.25/4	0.5/4	≤0.25/4 to 1/4
wzi50 (*n* = 7)	≤0.25/4	0.5/4	≤0.25/4 to 0.5/4
wzi83 (*n* = 6)	≤0.25/4	1/4	≤0.25/4 to 1/4
wzi154 (*n* = 4)	≤0.25/4	1/4	≤0.25/4 to 1/4
Wild-type *ompK35* and *ompK36* (*n* = 9)	≤0.25/4	≤0.25/4	≤0.25/4 to ≤0.25/4
ompK35 mutation only (*n* = 25)	≤0.25/4	0.5/4	≤0.25/4 to 1/4
ompK35 and *ompK36* mutations (*n* = 10)	≤0.25/4	1/4	≤0.25/4 to 1/4
KPC-3-expressing *K. pneumoniae* (*n* = 48)	≤0.25/4	0.5/4	≤0.25/4 to 1/4
ESBL (*n* = 15)	≤0.25/4	0.5/4	≤0.25/4 to 0.5/4
ST258 (*n* = 42)	≤0.25/4	0.5/4	≤0.25/4 to 1/4
Non-ST258 (*n* = 6)	≤0.25/4	4/4	≤0.25/4 to ≤0.25/4
wzi29 (*n* = 1)	0.5/4	0.5/4	0.5/4 to 0.5/4
wzi50 (*n* = 1)	≤0.25/4	≤0.25/4	≤0.25/4 to ≤0.25/4
wzi83 (*n* = 1)	0.5/4	0.5/4	0.5/4 to 0.5/4
wzi154 (*n* = 39)	≤0.25/4	0.5/4	≤0.25/4 to 1/4
Wild-type *ompK35* and *ompK36* (*n* = 6)	≤0.25/4	≤0.25/4	≤0.25/4 to ≤0.25/4
ompK35 mutation only (*n* = 20)	≤0.25/4	0.5/4	≤0.25/4 to 0.5/4
ompK35 and *ompK36* mutations (*n* = 21)	≤0.25/4	0.5/4	≤0.25/4 to 1/4

[a]The CLSI interpretive criteria for imipenem were applied to imipenem-relebactam.

imipenem-relebactam, whereas 90 (98%) KPC-expressing *K. pneumoniae* isolates were susceptible to ceftazidime-avibactam. Of note, the 2 *K. pneumoniae* isolates that were resistant to ceftazidime-avibactam were both KPC-3 expressing, with coexpression of 2 additional β-lactamases (wild-type TEM and wild-type SHV). In addition, the ceftazidime-avibactam-resistant isolates included 1 isolate with an *ompK35* mutation alone and another with both *ompK35* and *ompK36* mutations.

The *in vitro* activities of imipenem-relebactam and ceftazidime-avibactam against KPC-expressing *K. pneumoniae* isolates were further stratified by KPC allele, ESBL status, ST258 status, *wzi* allele, and mutation status for *ompK35* and *ompK36* outer membrane porin genes (Tables 1 and 2; also see Tables S6 to S9). There was no difference in imipenem-relebactam MIC values between KPC-2-expressing *K. pneumoniae* isolates and KPC-3-expressing *K. pneumoniae* isolates (MIC_{90} of 0.5/4 μg/ml for both; $P = 0.58$). Coharboring an ESBL gene did not increase imipenem-relebactam MICs. Additionally, the *in vitro* activity of imipenem-relebactam was not affected by *wzi* allele status or ST. However, outer membrane porin mutations had an impact on imipenem-relebactam MIC values (Table 1; also see Table S8), with strains harboring mutations in *ompK35* and *ompK36* having increased MIC values, compared to wild-type isolates (MIC_{90} values of 1/4 μg/ml versus ≤0.25/4 μg/ml; $P = 0.023$). No statistically significant increases in MIC values were observed for isolates with *ompK35* mutations only ($P = 0.14$). Unlike findings for ceftazidime-avibactam (Table S6), the KPC allelic variant had minimal impact on the effect of outer membrane porin mutations on imipenem-relebactam MIC values.

TABLE 2 Imipenem and imipenem-relebactam MIC values for OXA-48-like isolates stratified by ESBL status, OXA-like enzyme, and mutations in *ompK35* and *ompK36* outer membrane porin genes[a]

Drug and isolate type	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)
Imipenem			
OXA-48-like (*n* = 20)	8	≥32	8 to ≥32
ESBL (*n* = 16)	8	≥32	0.5/4 to ≥32
OXA-48 (*n* = 8)	16	≥32	≤0.25/4 to ≥32
OXA-163 (*n* = 1)	≤0.25	≤0.25	≤0.25 to ≤0.25
OXA-181 (*n* = 8)	4	≥32	4 to ≥32
OXA-232 (*n* = 3)	≥32	≥32	16 to ≥32
OXA-48-like-expressing *K. pneumoniae* (*n* = 14)	16	≥32	≤0.25 to ≥32
Wild-type *ompK35* and *ompK36* (*n* = 2)	16	≥32	16 to ≥32
ompK35 mutation only (*n* = 9)	8	≥32	≤0.25 to ≥32
ompK36 mutation only (*n* = 2)	4	≥32	4 to ≥32
ompK35 and *ompK36* mutations (*n* = 1)	16	16	16 to 16
Imipenem-relebactam			
OXA-48-like (*n* = 20)	4/4	≥32/4	≤0.25/4 to ≥32/4
ESBL (*n* = 16)	4/4	≥32/4	0.5/4 to ≥32/4
OXA-48 (*n* = 8)	4/4	≥32/4	≤0.25/4 to ≥32/4
OXA-163 (*n* = 1)	0.5/4	0.5/4	0.5/4 to 0.5/4
OXA-181 (*n* = 8)	4/4	≥32/4	2/4 to ≥32/4
OXA-232 (*n* = 3)	8/4	16/4	8/4 to 16/4
OXA-48-like-expressing *K. pneumoniae* (*n* = 14)	8/4	≥32/4	0.5/4 to ≥32/4
Wild-type *ompK35* and *ompK36* (*n* = 2)	8/4	≥32/4	≤0.25/4 to ≥32/4
ompK35 mutation only (*n* = 9)	4/4	≥32/4	0.5/4 to ≥32/4
ompK36 mutation only (*n* = 2)	4/4	≥32/4	4/4 to ≥32/4
ompK35 and *ompK36* mutations (*n* = 1)	4/4	4/4	4/4 to 4/4

[a]The CLSI interpretive criteria for imipenem were applied to imipenem-relebactam.

In summary, both imipenem-relebactam and ceftazidime-avibactam were effective against KPC-expressing *K. pneumoniae* CRE isolates, while only ceftazidime-avibactam was effective against OXA-48-like CRE isolates. Elevation of imipenem-relebactam MIC values was observed only among KPC-expressing *K. pneumoniae* isolates harboring both *ompK35* and *ompK36* mutations, although isolates with elevated MIC values were generally still considered susceptible when CLSI interpretive criteria for imipenem were applied.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00672-19.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

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