AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

SUSCEPTIBILITY

In Vitro Activity of Imipenem-Relebactam and Ceftolozane-Tazobactam against Resistant Gram-Negative Bacilli

Suzannah M. Schmidt-Malan,[a] Avisya J. Mishra,[a] Ammara Mushtaq,[a] Cassandra L. Brinkman,[a] Robin Patel[a,b]

[a]Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA

[b]Division of Infectious Diseases, Department of Medicine, Mayo Clinic, Rochester, Minnesota, USA

ABSTRACT Understanding which antimicrobial agents are likely to be active against Gram-negative bacilli can guide selection of antimicrobials for empirical therapy as mechanistic rapid diagnostics are adopted. In this study, we determined the MICs of a novel β-lactam–β-lactamase inhibitor combination, imipenem-relebactam, along with ceftolozane-tazobactam, imipenem, ertapenem, meropenem, ceftriaxone, and cefepime, against 282 drug-resistant isolates of Gram-negative bacilli. For isolates harboring bla_{KPC} ($n = 110$), the addition of relebactam to imipenem lowered the MIC_{50}/MIC_{90} from 16/>128 μg/ml for imipenem alone to 0.25/1 μg/ml. For isolates harboring bla_{CTX-M} ($n = 48$), the MIC_{50}/MIC_{90} of ceftolozane-tazobactam were 0.5/16 μg/ml (83% susceptible). For isolates harboring bla_{CMY-2} ($n = 17$), the MIC_{50}/MIC_{90} of ceftolozane-tazobactam were 4/8 μg/ml (47% susceptible). Imipenem-relebactam was active against most KPC-producing (but not NDM- or IMP-producing) *Enterobacteriaceae* and is an encouraging addition to the present antibiotic repertoire.

KEYWORDS antimicrobial resistance, Gram-negative bacilli

Multidrug-resistant Gram-negative bacilli are an increasing health care concern. Over the past decade, there has been a proliferation of resistance mechanisms, accompanied by an increase in overall levels of resistance (1, 2). This poses a challenge for treatment of infectious diseases due to the limitations in the activity of some currently available antimicrobial agents (3–6). In addition, newer agents are typically not active against all multidrug-resistant Gram-negative bacilli; their activity is influenced by the resistance mechanism(s) present. Of particular challenge are the *Enterobacteriaceae* and *Pseudomonas aeruginosa*, many isolates of which have developed resistance to target antimicrobials through acquisition of β-lactamases, including carbapenemases, extended-spectrum β-lactamases (ESBLs), and plasmid-mediated AmpC production (7–12). Additionally, mutations leading to loss of outer membrane porins or upregulation of efflux pumps can confer β-lactam resistance (2, 4, 13).

As multidrug-resistant Gram-negative bacilli often harbor β-lactamases, β-lactamase inhibitors can be used to restore activity of β-lactam antibiotics; however, their ability to do so depends on the particular β-lactamase present, alongside the activity of the individual β-lactam (14, 15).

Ceftolozane-tazobactam is a cephalosporin–β-lactamase inhibitor combination that is FDA cleared/approved for treatment of complicated intra-abdominal infections and complicated urinary tract infections caused by *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *P. aeruginosa*, as well as for treatment of complicated intra-abdominal infections caused by *Enterobacter cloacae* and *Klebsiella oxytoca* (16, 17). Ceftolozane-tazobactam is not active against serine carbapenemases such as *K. pneumoniae* carbapenemase (KPC) or against metallo-β-lactamases (8).

Imipenem-relebactam is an investigational carbapenem–β-lactamase inhibitor com-

Received 16 March 2018 **Returned for modification** 1 April 2018 **Accepted** 7 May 2018

Accepted manuscript posted online 14 May 2018

Citation Schmidt-Malan SM, Mishra AJ, Mushtaq A, Brinkman CL, Patel R. 2018. *In vitro* activity of imipenem-relebactam and ceftolozane-tazobactam against resistant Gram-negative bacilli. Antimicrob Agents Chemother 62:e00533-18. https://doi.org/10.1128/AAC.00533-18.

Address correspondence to Robin Patel, patel.robin@mayo.edu.

bination; relebactam is a diazabicyclooctane non-β-lactam β-lactamase inhibitor, in the same category as avibactam. However, although relebactam and avibactam are similar in chemical structure, relebactam has an additional piperidine ring. Relebactam shows activity against Ambler classes A and C β-lactamases; when paired with imipenem against KPC-producing *K. pneumoniae*, MIC values have been reported to decrease 64-fold (3, 7).

With the emergence of multidrug-resistant Gram-negative bacilli and the multitude of mechanisms by which resistance is affected, predicting the activity of novel antimicrobial agents using emerging diagnostic tools capable of identifying resistance genes and mutations is becoming increasingly clinically important. In this study, 282 drug-resistant Gram-negative bacilli, characterized using molecular methods, were tested for susceptibility to imipenem-relebactam, with ceftolozane-tazobactam, imipenem, ertapenem, meropenem, ceftriaxone, and cefepime studied as comparators.

RESULTS

Cumulative MICs for the study isolates are shown in Tables 1 and 2.

Carbapenemase gene-negative isolates. There were 48 isolates harboring genes for cefotaxime-hydrolyzing β-lactamase (CTX-M) and 17 harboring genes for cephamycin-hydrolyzing β-lactamase (CMY-2) (see Table S1 in the supplemental material).

The MIC_{50}/MIC_{90} of the CTX-M-positive isolates were 0.125/0.25 μg/ml for both imipenem-relebactam and imipenem. All CTX-M-positive isolates were susceptible to imipenem (as well as to imipenem-relebactam if using the imipenem breakpoint). Eighty-three percent of these isolates were susceptible to ceftolozane-tazobactam, while 12 and 0% were susceptible to cefepime and ceftriaxone, respectively. The MIC_{50}/MIC_{90} for the same isolates were 0.5/16, >128/>128, and >128/>128 μg/ml for ceftolozane-tazobactam, cefepime, and ceftriaxone, respectively (Table 1).

Eighty-eight percent of CMY-2-positive isolates were susceptible to imipenem (as well as to imipenem-relebactam if using the imipenem breakpoint). The MIC_{50}/MIC_{90} for the CMY-2-positive isolates were 0.25/0.5 and 0.25/1 μg/ml for imipenem-relebactam and imipenem, respectively. Forty-seven percent of CMY-2-positive isolates were susceptible to ceftolozane-tazobactam, 88% were susceptible to cefepime, and 12% were susceptible to ceftriaxone. The MIC_{50}/MIC_{90} for the CMY-2-positive isolates were 4/8, 0.25/2, and 64/128 μg/ml for ceftolozane-tazobactam, cefepime, and ceftriaxone, respectively (Table 1).

For the other resistance mechanisms tested, there were fewer than 10 isolates per group, so MIC_{50}/MIC_{90} values were not calculated. Nonetheless, all isolates harboring cefoxitin-hydrolyzing β-lactamase (FOX-5), SHV, TEM, TEM plus SHV (11 of which were ESBL producers as determined by clavulanate disc augmentation), or other combined mechanisms of resistance were susceptible to imipenem, meropenem, and ertapenem, and the addition of relebactam to imipenem did not change their MIC values compared to those for imipenem alone (Table S1). For ceftolozane-tazobactam, there were some differences noted compared to the other cephalosporins. The FOX-5 isolate was susceptible to ceftolozane-tazobactam and cefepime and intermediate to ceftriaxone. The SHV-positive isolates were all resistant to ceftriaxone, 3 of 6 were resistant to cefepime, and 4 of 6 were resistant to ceftolozane-tazobactam. For the TEM-positive isolates, 7/8 and 4/8 were resistant to ceftriaxone and cefepime, respectively, while 4, 2, and 2 were susceptible, intermediate, and resistant, respectively, to ceftolozane-tazobactam. Thirty-three percent of dually TEM- and SHV-positive isolates were susceptible to ceftolozane-tazobactam, while 25 and 0% were susceptible to cefepime and ceftriaxone, respectively. For the 19 isolates with other combined mechanisms of resistance, 17, 12, and 9 were resistant to ceftriaxone, cefepime, and ceftolozane-tazobactam, respectively.

Carbapenemase gene-positive isolates. The carbapenemase gene-positive isolates included 31 positive for New Delhi metallo-β-lactamase (NDM), 11 positive for imipenem metallo-β-lactamase (IMP), and 110 positive for KPC (Table S2). All NDM-

TABLE 1 Cumulative MIC results for CTX-M and CMY-2 gene-positive isolates for all antimicrobials tested[a]

Type of isolate and drug	No. of isolates (cumulative %) inhibited at specified concn (μg/ml)																		MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% S	% I	% R
	0.0018	0.0037	0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128					
CTX-M gene-positive isolates (n = 48)																							
Imipenem (4 μg/ml relebactam)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.3)	16 (39.6)	20 (81.3)	6 (93.8)	2 (97.9)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0.125	0.25	100.0[b]	0.0[b]	0.0[b]
Imipenem	0 (0.0)	0 (0.0)	0 (0.0)	2 (4.2)	3 (10.4)	8 (27.1)	26 (81.3)	7 (95.8)	2 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0.125	0.25	100.0	0.0	0.0
Ertapenem	1 (2.1)	0 (0.0)	5 (12.5)	10 (33.3)	12 (58.3)	6 (70.8)	3 (77.1)	5 (87.5)	4 (95.8)	1 (97.9)	0 (100.0)	1 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0.03	0.5	95.8	2.1	2.1
Meropenem	0 (0.0)	2 (4.2)	4 (12.5)	25 (64.6)	11 (87.5)	5 (97.9)	0 (97.9)	1 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0.015	0.06	100.0	0.0	0.0
Ceftolozane (4 μg/ml tazobactam)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.3)	17 (41.7)	9 (60.4)	8 (77.1)	3 (83.3)	0 (83.3)	2 (87.5)	3 (93.8)	1 (95.8)	0 (95.8)	0 (95.8)	2 (100.0)	0.5	16	83.3	0.0	16.7
Cefepime	0 (0.0)	0 (0.0)	2 (4.2)	0 (4.2)	0 (4.2)	0 (4.2)	1 (6.3)	0 (6.3)	0 (6.3)	0 (6.3)	3 (12.5)	1 (14.6)	0 (14.6)	7 (29.2)	1 (31.3)	0 (31.3)	2 (35.4)	31 (100.0)	>128	>128	12.5	NA	85.4
Ceftriaxone	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.1)	1 (4.2)	0 (4.2)	1 (6.3)	1 (8.3)	1 (10.4)	43 (100.0)	>128	>128	0.0	0.0	100.0
CMY-2 gene-positive isolates (n = 17)																							
Imipenem (4 μg/ml relebactam)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.8)	0 (11.7)	6 (47.0)	5 (76.5)	2 (88.2)	0 (88.2)	0 (88.2)	2 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0.25	0.5	88.2[b]	0.0[b]	11.8[b]
Imipenem	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (17.6)	6 (52.9)	3 (70.6)	3 (88.2)	0 (88.2)	2 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0.25	1	88.2	0.0	11.8
Ertapenem	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (29.4)	1 (35.3)	2 (47.0)	3 (64.7)	3 (82.4)	1 (88.2)	0 (88.2)	2 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0.25	1	82.4	5.9	11.8
Meropenem	0 (0.0)	0 (0.0)	0 (0.0)	9 (52.9)	5 (82.3)	1 (88.2)	2 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0.015	0.06	100.0	0.0	0.0
Ceftolozane (4 μg/ml tazobactam)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.9)	3 (23.5)	2 (35.3)	2 (47.0)	4 (70.6)	3 (88.2)	2 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	4	8	47.0	23.5	29.4
Cefepime	0 (0.0)	0 (0.0)	1 (5.9)	2 (17.6)	0 (17.6)	0 (17.6)	2 (29.4)	5 (58.8)	2 (70.6)	1 (76.5)	2 (88.2)	0 (88.2)	0 (88.2)	0 (88.2)	1 (94.1)	0 (94.1)	0 (94.1)	1 (100.0)	0.25	2	88.2	NA	11.8
Ceftriaxone	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.8)	0 (118)	0 (11.8)	0 (11.8)	0 (11.8)	0 (11.8)	0 (11.8)	1 (17.6)	4 (41.2)	1 (47.0)	4 (70.6)	4 (94.1)	1 (100.0)	64	128	11.8	0.0	88.2

[a]S, susceptible; I, intermediate; R, resistant; NA, not applicable.

[b]Using imipenem breakpoints.

TABLE 2 Cumulative MIC results for NDM, IMP, and KPC gene-positive isolates for all antimicrobial agents tested[a]

Type of isolate and drug	No. of isolates (cumulative %) inhibited at specified concn (μg/ml)																		MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% S	% I	% R
	0.0018	0.0037	0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128					
NDM gene-positive isolates (n = 31)																							
Imipenem (4 μg/ml relebactam)						0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.2)	4 (16.1)	10 (48.4)	9 (77.4)	6 (96.7)	1 (100.0)	32	128	0[b]	0[b]	100[b]
Imipenem						0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.2)	4 (16.1)	12 (54.8)	7 (77.4)	5 (93.5)	2 (100.0)	32	128	0	0	100
Ertapenem						0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.2)	3 (12.9)	9 (41.9)	10 (74.2)	8 (100.0)	128	>128	0	0	100
Meropenem						0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (12.9)	4 (25.8)	9 (54.8)	10 (87.1)	4 (100.0)	64	>128	0	0	100
Ceftolozane (4 μg/ml tazobactam)						0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	31 (100.0)	>128	>128	0	0	100
Cefepime						0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (6.4)	2 (12.9)	27 (100.0)	>128	>128	0	NA	100
Ceftriaxone						0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	31 (100.0)	>128	>128	0	0	100
IMP gene-positive isolates (n = 11)																							
Imipenem (4 μg/ml relebactam)					0 (0.0)	1 (9.1)	0 (9.1)	0 (9.1)	0 (9.1)	1 (18.2)	1 (27.3)	3 (54.5)	1 (63.6)	1 (72.7)	0 (72.7)	0 (72.7)	2 (90.9)	1 (100.0)	4	128	18.2[b]	9.1[b]	72.7[b]
Imipenem					0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)	1 (18.2)	1 (27.3)	2 (45.5)	2 (63.6)	1 (72.7)	0 (72.7)	0 (72.7)	2 (90.9)	1 (100.0)	8	128	18.2	9.0	72.8
Ertapenem					0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (18.2)	1 (27.3)	0 (27.3)	4 (63.6)	0 (63.6)	1 (72.7)	1 (81.8)	2 (100.0)	16	>128	0	0	100
Meropenem					0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)	2 (27.3)	0 (27.3)	0 (27.3)	3 (54.5)	1 (63.6)	1 (72.7)	1 (81.8)	2 (100.0)	0 (100.0)	8	128	27.3	0	72.7
Ceftolozane (4 μg/ml tazobactam)					0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)	0 (9.1)	0 (9.1)	1 (18.2)	9 (100.0)	>128	>128	0	0	100
Cefepime					0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)	2 (27.3)	1 (36.4)	1 (45.5)	0 (45.5)	6 (100.0)	>128	>128	0	NA	91.9
Ceftriaxone					0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)	2 (27.3)	8 (100.0)	>128	>128	0	0	100
KPC gene-positive isolates (n = 110)																							
Imipenem (4 μg/ml relebactam)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	5 (5.4)	15 (18.9)	24 (40.5)	20 (58.6)	22 (78.4)	14 (90.9)	4 (94.6)	1 (95.5)	1 (96.4)	2 (98.2)	1 (99.1)	0 (99.1)	0 (99.1)	1 (100.0)	0.25	1	90.9[b]	3.6[b]	5.4[b]
Imipenem	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.8)	1 (2.7)	2 (4.5)	3 (7.2)	16 (21.6)	15 (35.1)	20 (53.2)	20 (71.2)	11 (81.1)	7 (87.4)	14 (100.0)	16	>128	4.5	2.7	92.8
Ertapenem	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	0 (1.0)	0 (1.0)	0 (1.0)	2 (2.7)	1 (3.6)	4 (7.2)	9 (15.3)	13 (27.0)	16 (41.4)	20 (59.5)	22 (79.3)	23 (100.0)	64	>128	1.8	1.0	97.2
Meropenem	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	1 (1.8)	0 (1.8)	0 (1.8)	1 (2.7)	3 (5.4)	3 (8.1)	11 (18.0)	13 (29.7)	13 (41.4)	23 (62.2)	9 (70.3)	3 (73.0)	30 (100.0)	32	>128	5.4	2.7	91.9
Ceftolozane (4 μg/ml tazobactam)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	2 (2.7)	1 (3.6)	1 (4.5)	1 (5.4)	6 (10.8)	13 (22.5)	21 (41.4)	37 (74.8)	28 (100.0)	128	>128	3.6	1	95.4
Cefepime	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	0 (1.0)	1 (1.8)	1 (2.7)	4 (6.3)	7 (12.6)	9 (20.7)	6 (26.1)	9 (34.2)	10 (43.2)	63 (100.0)	>128	>128	2.7	NA	97.3
Ceftriaxone	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	1 (1.8)	0 (1.8)	1 (2.7)	1 (3.6)	0 (3.6)	6 (9.0)	8 (16.2)	93 (100.0)	>128	>128	1.8	1.0	97.2

[a]S, susceptible; I, intermediate; R, resistant; NA, not applicable.

[b]Using imipenem breakpoints.

positive isolates were resistant to imipenem, and their MIC_{50}/MIC_{90} were 32/128 μg/ml for both imipenem and imipenem-relebactam; if using the imipenem breakpoint, all isolates would be considered resistant to imipenem-relebactam. All NDM-positive isolates were also resistant to ceftolozane-tazobactam, cefepime, and ceftriaxone, with MIC_{50} and MIC_{90} values all being >128 μg/ml (Table 2). Eighteen percent of IMP-positive isolates were susceptible to imipenem (as well as to imipenem-relebactam if using the imipenem breakpoint). The MIC_{50}/MIC_{90} for the IMP isolates were 4/128 and 8/128 μg/ml for imipenem-relebactam and imipenem, respectively. All IMP-positive isolates were resistant to ceftolozane-tazobactam, with MIC_{50} and MIC_{90} values of >128 μg/ml; they exhibited 8 and 0% susceptibility to cefepime and ceftriaxone, respectively (Table 2). Five percent of the KPC-positive isolates were susceptible to imipenem; 91% would be considered susceptible to imipenem-relebactam if using the imipenem breakpoints. The MIC_{50}/MIC_{90} for the KPC-positive isolates were 0.25/1 and 16/>128 μg/ml for imipenem-relebactam and imipenem, respectively. Three percent and 2% of the KPC-positive isolates were susceptible to cefepime and ceftriaxone, respectively, and 4% were susceptible to ceftolozane-tazobactam. The MIC_{50}/MIC_{90} for ceftolozane-tazobactam for the KPC-positive isolates were 128/>128 μg/ml (Table 2).

All imipenem-hydrolyzing β-lactamase (IMI)-positive isolates were resistant to imipenem and susceptible to ceftolozane-tazobactam, cefepime, and ceftriaxone; addition of relebactam to imipenem lowered the MIC values up to 6 doubling dilutions compared to those for imipenem alone (Table S2). For oxacillin-hydrolyzing β-lactamase (OXA)-positive isolates (including OXA-48, -181, and -232), addition of relebactam to imipenem did not change MIC values compared to those for imipenem alone; isolates which were resistant to ceftriaxone and cefepime were also resistant to ceftolozane-tazobactam. One isolate was susceptible to ceftriaxone but resistant to ceftolozane-tazobactam and cefepime. For the OXA-48-positive isolates, 4 of 8 had imipenem-relebactam MICs of ≤1 μg/ml. In the case of two *Serratia marcescens* enzyme (SME)-positive isolates, the addition of relebactam to imipenem dropped the MIC from 128 μg/ml for imipenem alone to 0.5 μg/ml for one and from >128 μg/ml to 16 for the other; it did not change for the MIC of the third SME-positive isolate. Finally, for the Verona integrin-encoded metallo-β-lactamase (VIM)-positive isolates, all of which were resistant to imipenem, ceftolozane-tazobactam, cefepime, and ceftriaxone, the addition of relebactam to imipenem did not change the MICs compared to those for imipenem alone (Table S2).

DISCUSSION

For the isolates which had tested positive for CTX-M or CMY-2, the addition of relebactam to imipenem did not change the MIC_{50}/MIC_{90}; 100 and 88% of these isolates, respectively, were susceptible to imipenem alone, as expected (1). The addition of tazobactam to ceftolozane inhibited most CTX-M-positive isolates, rendering 83% susceptible. These results are not unexpected given that tazobactam inhibits most class A β-lactamases, including common ESBL enzymes, such as CTX-M (3, 11). There are CTX-M variants that have amino acid substitutions which improve recognition of ceftazidime (namely, D240G and P167S/T) but may result in attenuated bioactivity of cefotaxime and/or cefepime (18); this could explain the high rate of resistance to cefepime noted.

For isolates harboring CMY-2, the addition of tazobactam to ceftolozane appears to slightly increase the susceptibility compared to that for ceftriaxone, although fewer than half were susceptible to ceftolozane-tazobactam. This is not surprising given that tazobactam is only a moderate inhibitor of class C β-lactamases and has strain-dependent activity (19). For isolates harboring CMY-2, first- through fourth-generation cephalosporins are expected to be hydrolyzed. However, cefepime may be active (1), which is consistent with our findings.

The addition of relebactam to imipenem lowered the MIC_{50}/MIC_{90} from 16/>128 μg/ml to 0.25/1 μg/ml for the KPC-positive isolates, 93% of which were resistant to imipenem alone. Currently, there are no established breakpoints for imipenem-

relebactam, but using the breakpoint for imipenem alone, relebactam restored the activity of imipenem in isolates testing positive for KPC. As expected, this increased susceptibility to imipenem-relebactam compared to imipenem alone was not noted for the NDM-positive or IMP-positive isolates; relebactam overcomes class A and C (20) and not class B β-lactamases, which include NDM and IMP (1). Among the NDM-, IMP-, and KPC-harboring isolates, more than 90% were resistant to the cephalosporins tested (ceftolozane-tazobactam, cefepime, and ceftriaxone), which is expected given that class A carbapenemases and class C β-lactamases hydrolyze first- through fourth-generation cephalosporins (1, 8, 21). Our findings agree with a prior study showing that ceftolozane-tazobactam is inactive against *Enterobacteriaceae* harboring class A serine carbapenemases (e.g., KPC) and class B metallo-β-lactamases (e.g., NDM, IMP, and VIM) (8). Interestingly, 4 of the 8 OXA-48-positive isolates had imipenem-relebactam MICs of $\leq$1 μg/ml. This has not been well described in the literature thus far, but one study by Livermore et al. tested imipenem-relebactam against 5 *K. pneumoniae* OXA-48-positive isolates and found that 3 out of 5 isolates had MICs of $\leq$1 μg/ml (20).

One limitation to our study is that although the study isolates were fairly well characterized, many of the resistance mechanisms were present in only a limited number of isolates. We can deduce trends from only some groups of isolates and are not able to provide mechanism-specific MIC_{50}/MIC_{90} data for all resistance types. A second limitation is that resistance in Gram-negative bacilli is complex and we evaluated the impact of only selected β-lactamases. For example, the KPC- and NDM-positive isolates in this study were not further characterized, and there could be other β-lactamases present. We did not evaluate other potentially concomitant mechanisms of resistance, such as outer membrane permeability and efflux, which, when present in combination with β-lactamases, can affect *in vitro* activity. Our identification of isolates with AmpCs that were nonsusceptible to ceftolozane-tazobactam suggests the presence of other resistance mechanisms (e.g., porin mutations). *K. pneumoniae* IDRL-10648, reported only as having a non-ESBL SHV-1, must have additional resistance mechanisms because the MICs of ceftolozane-tazobactam, ceftriaxone, and cefepime were very high. Another limitation is that at the time of this writing, there are no established breakpoints for imipenem-relebactam.

Overall, results of this study show that imipenem-relebactam is active against most KPC-positive (but not NDM- or IMP-positive) *Enterobacteriaceae*. Imipenem-relebactam is a promising addition to the existing antibiotic armamentarium; however, clinicians will need to be aware of geographical epidemiology and the genetic makeup of strains.

MATERIALS AND METHODS

Bacterial strains. A total of 282 previously characterized isolates of Gram-negative bacilli collected from across the United States, Canada, and Singapore were studied. The isolates exhibited one or more of the following resistance mechanisms previously or during this study genotypically characterized by PCR with or without sequencing (9, 12, 22–31): TEM, SHV, CMY, IMP, NDM, KPC, OXA, VIM, CTX-M, SME, FOX, and/or IMI. One hundred five isolates were carbapenemase gene negative and included *E. coli* (74), *K. pneumoniae* (28), *Morganella morganii* (2), and *Enterobacter cloacae* complex (1) (Table S3). A total of 177 harbored carbapenemase genes, including 4 *Citrobacter freundii*, 3 *Citrobacter koseri*, 1 *Citrobacter sedlakii*, 3 *Enterobacter aerogenes*, 20 *E. cloacae*, 15 *E. coli*, 119 *K. pneumoniae*, 4 *P. aeruginosa*, 1 *P. mirabilis*, 2 *Providencia stuartii*, and 5 *Serratia marcescens* isolates (Table S4). Six quality control strains were tested with each trial: *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 35218, and *K. pneumoniae* ATCC 700603. All isolates were stored in MicroBank vials (Pro-Lab Diagnostics, Round Rock, TX) at −80°C.

Antimicrobial agent preparation. Seven antibiotics, including cefepime, ceftriaxone, and meropenem (provided by USP, Rockville, MD) and ertapenem, imipenem, imipenem-relebactam, and ceftolozane-tazobactam (provided by Merck & Co., Inc.) were studied. Stock solutions of each antibiotic were prepared according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (32, 33). Prepared stock solutions were aliquoted into 1.5-ml microcentrifuge tubes and stored at −80°C.

Broth microdilution susceptibility testing. Bacteria were grown for 18 to 20 h on BBL Trypticase soy agar with 5% sheep blood (Becton Dickinson, Franklin Lakes, NJ) at 37°C and then subcultured for study of F_2 generation colonies. MICs were determined by broth microdilution, using the same inoculum for all antimicrobials, following CLSI guidelines (33). Antimicrobial concentrations tested ranged from 0.0018 to 128 μg/ml, in 2-fold serial dilutions, using a constant concentration of 4 μg/ml of relebactam and tazobactam when combined with imipenem or ceftolozane, respectively. Plates were incubated for

16 to 20 h at 37°C, and the MIC was recorded as the first well with no growth. The MIC_{50}/MIC_{90}, cumulative MICs, and percent of isolates that were susceptible, intermediate, and resistant for each antimicrobial agent were interpreted using CLSI breakpoints (33).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00533-18.

SUPPLEMENTAL FILE 1, DOCX file, 0.08 MB.

ACKNOWLEDGMENTS

Research reported in this publication was supported by Merck & Co.

We acknowledge Kerryl E. Greenwood-Quaintance, M.S., Melissa J. Karau, Audrey N. Schuetz, Peggy C. Kohner, Nicolynn C. Cole, and Scott A. Cunningham for technical advice. We thank Mayo Clinic Clinical Bacteriology Lab, Donna J. Hata from Mayo Clinic in Jacksonville, FL, James R. Johnson from the VA Medical Center in Minneapolis, MN, Hennepin County Medical Center in Minneapolis, MN, Mary K. Hayden and Karen Lolans from Rush University Medical Center, and Paul C. Schreckenberger from Loyola, both in Chicago, IL, Patricia J. Simner with the Canadian Antimicrobial Resistance Alliance and the CANWARD study, George G. Zhanel and Daryl J. Hoban from the University of Manitoba, Partha Pratim De, Sanjay Ryan Menon, and Shawn Vasoo from Tan Tock Seng Hospital, and Koh Tse Hsien from Singapore General Hospital in Singapore for isolates included in this study.

REFERENCES

1. Vasoo S, Barreto JN, Tosh PK. 2015. Emerging issues in Gram-negative bacterial resistance: an update for the practicing clinician. Mayo Clin Proc 90:395–403. https://doi.org/10.1016/j.mayocp.2014.12.002.
2. Bornet C, Davin-Regli A, Bosi C, Pages J-M, Bollet C. 2000. Imipenem resistance of *Enterobacter aerogenes* mediated by outer membrane permeability. J Clin Microbiol 38:1048–1052.
3. Syue LS, Chen YH, Ko WC, Hsueh PR. 2016. New drugs for the treatment of complicated intra-abdominal infections in the era of increasing antimicrobial resistance. Int J Antimicrob Agents 47:250–258. https://doi.org/10.1016/j.ijantimicag.2015.12.021.
4. Pavez M, Vieira C, de Araujo MR, Cerda A, de Almeida LM, Lincopan N, Mamizuka EM. 2016. Molecular mechanisms of membrane impermeability in clinical isolates of *Enterobacteriaceae* exposed to imipenem selective pressure. Int J Antimicrob Agents 48:78–85. https://doi.org/10.1016/j.ijantimicag.2016.04.016.
5. Giancola SE, Mahoney MV, Bias TE, Hirsch EB. 2016. Critical evaluation of ceftolozane-tazobactam for complicated urinary tract and intra-abdominal infections. Ther Clin Risk Manage 12:787–797.
6. Liscio JL, Mahoney MV, Hirsch EB. 2015. Ceftolozane/tazobactam and ceftazidime/avibactam: two novel beta-lactam/beta-lactamase inhibitor combination agents for the treatment of resistant Gram-negative bacterial infections. Int J Antimicrob Agents 46:266–271. https://doi.org/10.1016/j.ijantimicag.2015.05.003.
7. Lapuebla A, Abdallah M, Olafisoye O, Cortes C, Urban C, Landman D, Quale J. 2015. Activity of imipenem with relebactam against Gram-negative pathogens from New York City. Antimicrob Agents Chemother 59:5029–5031. https://doi.org/10.1128/AAC.00830-15.
8. Sucher AJ, Chahine EB, Cogan P, Fete M. 2015. Ceftolozane/tazobactam: a new cephalosporin and beta-lactamase inhibitor combination. Ann Pharmacother 49:1046–1056. https://doi.org/10.1177/1060028015593293.
9. Kohner PC, Robberts FJ, Cockerill FR, Patel R. 2009. Cephalosporin MIC distribution of extended-spectrum-β-lactamase-and pAmpC-producing *Escherichia coli* and *Klebsiella* species. J Clin Microbiol 47:2419–2425. https://doi.org/10.1128/JCM.00508-09.
10. Queenan AM, Bush K. 2007. Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev 20:440–458. https://doi.org/10.1128/CMR.00001-07.
11. Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. 2015. Antibiotic resistance and extended spectrum beta-lactamases: types, epidemiology and treatment. Saudi J Biol Sci 22:90–101. https://doi.org/10.1016/j.sjbs.2014.08.002.
12. Vasoo S, Cunningham SA, Cole NC, Kohner PC, Menon SR, Krause KM, Harris KA, De Partha P, Koh TH, Patel R. 2015. *In vitro* activity of ceftazidime-avibactam, aztreonam-avibactam and a panel of older and contemporary antimicrobial agents against carbapenemase-producing Gram-negative bacilli. Antimicrob Agents Chemother 59:7842–7846. https://doi.org/10.1128/AAC.02019-15.
13. Cejas D, Fernandez Canigia L, Quinteros M, Giovanakis M, Vay C, Lasciallandare S, Mutti D, Pagniez G, Almuzara M, Gutkind G, Radice M. 2012. Plasmid-encoded AmpC (pAmpC) in *Enterobacteriaceae*: epidemiology of microorganisms and resistance markers. Rev Argent Microbiol 44:182–186.
14. Bush K, Bradford PA. 2016. Beta-lactams and beta-lactamase inhibitors: an overview. Cold Spring Harbor Perspect Med 6:a025247. https://doi.org/10.1101/cshperspect.a025247.
15. Bush K. 2015. A resurgence of beta-lactamase inhibitor combinations effective against multidrug-resistant Gram-negative pathogens. Int J Antimicrob Agents 46:483–493. https://doi.org/10.1016/j.ijantimicag.2015.08.011.
16. Eckmann C, Solomkin J. 2015. Ceftolozane/tazobactam for the treatment of complicated intra-abdominal infections. Expert Opin Pharmacother 16:271–280. https://doi.org/10.1517/14656566.2015.994504.
17. Skalweit MJ. 2015. Profile of ceftolozane/tazobactam and its potential in the treatment of complicated intra-abdominal infections. Drug Des Devel Ther 9:2919–2925. https://doi.org/10.2147/DDDT.S61436.
18. D'Andrea MM, Arena F, Pallecchi L, Rossolini GM. 2013. CTX-M-type β-lactamases: a successful story of antibiotic resistance. Int J Med Microbiol 303:305–317. https://doi.org/10.1016/j.ijmm.2013.02.008.
19. Farrell DJ, Flamm RK, Sader HS, Jones RN. 2013. Antimicrobial activity of ceftolozane-tazobactam tested against *Enterobacteriaceae* and *Pseudomonas aeruginosa* with various resistance patterns isolated in U.S. Hospitals (2011-2012). Antimicrob Agents Chemother 57:6305–6310. https://doi.org/10.1128/AAC.01802-13.
20. Livermore DM, Warner M, Mushtaq S. 2013. Activity of MK-7655 combined with imipenem against *Enterobacteriaceae* and *Pseudomonas aeruginosa*. J Antimicrob Chemother 68:2286–2290.
21. Sacha P, Ostas A, Jaworowska J, Wieczorek P, Ojdana D, Ratajczak J, Tryniszewska E. 2009. The KPC type beta-lactamases: new enzymes that confer resistance to carbapenems in Gram-negative bacilli. Folia Histochem Cytobiol 47:537–543.
22. Balm M, La MV, Krishnan P, Jureen R, Lin R, Teo J. 2013. Emergence of *Klebsiella pneumoniae* co-producing NDM-type and OXA-181 carbapenemases. Clin Microbiol Infect 19:E421–E423. https://doi.org/10.1111/1469-0691.12247.
23. Cunningham SA, Noorie T, Meunier D, Woodford N, Patel R. 2013. Rapid

and simultaneous detection of genes encoding *Klebsiella pneumoniae* carbapenemase (bla_{KPC}) and New Delhi metallo-β-lactamase (bla_{NDM}) in Gram-negative bacilli. J Clin Microbiol 51:1269–1271. https://doi.org/10.1128/JCM.03062-12.
24. Koh TH, Cao D, Shan QY, Bacon A, Hsu L-Y, Ooi EE. 2013. Acquired carbapenemases in *Enterobactericeae* in Singapore, 1996–2012. Pathology 45:600–603. https://doi.org/10.1097/PAT.0b013e3283650b1e.
25. Poirel L, Héritier C, Tolün V, Nordmann P. 2004. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 48:15–22. https://doi.org/10.1128/AAC.48.1.15-22.2004.
26. Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo J-D, Nordmann P. 2000. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-β-lactamase and its plasmid-and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob Agents Chemother 44:891–897. https://doi.org/10.1128/AAC.44.4.891-897.2000.
27. Teo J, Kurup A, Lin R, Hsien K. 2013. Emergence of clinical *Klebsiella pneumoniae* producing OXA-232 carbapenemase in Singapore. New Microbes New Infect 1:13–15. https://doi.org/10.1002/2052-2975.4.
28. Teo J, Ngan G, Balm M, Jureen R, Krishnan P, Lin R. 2012. Molecular characterization of NDM-1 producing *Enterobacteriaceae* isolates in Singapore hospitals. Western Pac Surveill Response J 3:19. https://doi.org/10.5365/wpsar.2011.2.4.010.
29. Teo JW, La M-V, Krishnan P, Ang B, Jureen R, Lin RT. 2013. *Enterobacter cloacae* producing an uncommon class A carbapenemase, IMI-1, from Singapore. J Med Microbiol 62:1086–1088. https://doi.org/10.1099/jmm.0.053363-0.
30. Robberts F, Kohner P, Patel R. 2009. Unreliable extended-spectrum β-lactamase detection in the presence of plasmid-mediated AmpC in *Escherichia coli* clinical isolates. J Clin Microbiol 47:358–361. https://doi.org/10.1128/JCM.01687-08.
31. Cunningham SA, Vasoo S, Patel R. 2016. Evaluation of the Check-Points Check MDR CT103 and CT103 XL microarray kits by use of preparatory rapid cell lysis. J Clin Microbiol 54:1368–1371. https://doi.org/10.1128/JCM.03302-15.
32. Clinical and Laboratory Standards Institute. 2018. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 11th ed. CLSI document M07. Clinical and Laboratory Standards Institute, Wayne, PA.
33. Clinical and Laboratory Standards Institute. 2018. Performance standards for antimicrobial susceptibility testing, 28th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.