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Activity of Imipenem with Relebactam against Gram-Negative Pathogens from New York City

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Imipenem with relebactam was active against *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* spp., including *K. pneumoniae* carbapenemase (KPC)-producing isolates. Loss of OmpK36 in KPC-producing *K. pneumoniae* isolates affected the susceptibility of this combination. Enhanced activity was evident against *Pseudomonas aeruginosa*, including isolates with depressed *oprD* and increased *ampC* expression. However, the addition of relebactam to imipenem did not provide added benefit against *Acinetobacter baumannii*. The combination of imipenem with relebactam demonstrated activity against KPC-producing *Enterobacteriaceae* and multidrug-resistant *P. aeruginosa*.

The spread of carbapenemases in *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* has created therapeutic dilemmas for clinicians. In particular, the acquisition of metallo-β-lactamases and the class A enzyme *Klebsiella pneumoniae* carbapenemase (KPC) affords protection against virtually all β-lactam therapeutic agents (1). The worldwide dissemination of bacteria possessing bla_{KPC} has been especially striking (2). First reported in the northeastern United States in the 1990s, pathogens harboring this β-lactamase are now endemic in countries in Asia, South America, and Europe (2).

Novel β-lactamase inhibitors are being developed to restore the utility of β-lactam antibiotics against carbapenemase-producing pathogens (3, 4). Avibactam and relebactam are diazabicyclooctane inhibitors with activity against a wide spectrum of β-lactamases, including class A (extended-spectrum β-lactamases [ESBLs] and KPC) and class C (AmpC) enzymes (3, 4). In this study, we determined the activity of imipenem with relebactam against Gram-negative pathogens from medical centers in New York City.

Between November 2013 and January 2014, single patient isolates of *Escherichia coli*, *K. pneumoniae*, *Enterobacter* spp., *P. aeruginosa*, and *A. baumannii* were collected from 11 hospitals in Brooklyn and Queens, New York. Susceptibility tests were performed in a central research laboratory using the agar dilution method, and results were interpreted according to CLSI guidelines (5). Isolates of *E. coli* and *K. pneumoniae* were presumed to harbor ESBLs if they were not susceptible to ceftazidime and/or ceftriaxone and did not have bla_{KPC}. Imipenem was tested both with and without the presence of relebactam (fixed concentration of 4 μg/ml). For the purposes of this study, imipenem breakpoints were used to interpret susceptibility to imipenem plus relebactam. Cephalosporin-resistant isolates were tested by PCR for the presence of bla_{KPC}, $bla_{OXA\text{-}23\text{-}type}$, $bla_{OXA\text{-}24\text{-}type}$, bla_{VIM}, bla_{IMP}, and bla_{NDM} using previously described primers (6–9). Isolates of *K. pneumoniae* with bla_{KPC} and isolates of *A. baumannii* with $bla_{OXA23\text{-}type}$ underwent genetic fingerprinting by the repetitive element palindromic PCR (rep-PCR) method with the ERIC-2 primer, as described previously (6).

Susceptibility testing of imipenem with and without relebactam was also performed with a collection of previously characterized isolates of *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* (10–13). The expression of genes encoding β-lactamases, efflux pumps, and porins was correlated with the MICs for imipenem with relebactam.

Surveillance study results. A total of 2,778 isolates of *E. coli* were gathered during the 3-month surveillance study. Susceptibilities are presented in Table 1. Of the bla_{KPC}-negative isolates, 383 were considered to have ESBLs and all were susceptible to imipenem. The imipenem MIC_{50} and MIC_{90} values for the ESBL-producing isolates were 0.25 and 0.5 μg/ml, respectively; with the addition of relebactam, the corresponding values were 0.25 and 0.25 μg/ml. Five isolates harbored bla_{KPC}. For these 5 isolates, the imipenem MICs ranged from 0.5 to >32 μg/ml. With the addition of relebactam, the MICs decreased to 0.12 to 0.5 μg/ml.

A total of 891 isolates of *K. pneumoniae* were collected (Table 1). Of the bla_{KPC}-negative isolates, 185 were considered ESBL producers. All of the ESBL producers were susceptible to imipenem, with MIC_{50} and MIC_{90} values of 0.25 and 0.5 μg/ml, respectively. With the addition of relebactam, the imipenem MIC_{50} and MIC_{90} values were 0.25 and 0.5 μg/ml, respectively. For 111 isolates that harbored bla_{KPC}, the imipenem MIC_{50} and MIC_{90} values were 16 and >16 μg/ml, respectively. With the addition of relebactam, the MIC_{50} and MIC_{90} values decreased to 0.25 and 1 μg/ml, respectively; three isolates had MICs of 2 μg/ml (intermediate resistance to imipenem). Twenty-three isolates with bla_{KPC}, from 11 different hospitals, underwent fingerprinting. Eight rep-PCR types were identified, including 12 isolates that belonged to one clone (data not shown).

There were 211 *Enterobacter* isolates (Table 1), including 90 *Enterobacter aerogenes* isolates and 120 *Enterobacter cloacae* iso-

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TABLE 1 Susceptibility results for *Enterobacteriaceae*, *P. aeruginosa*, and *A. baumannii* isolates collected in surveillance study

Species and drug(s)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)	% susceptible
E. coli ($n = 2,778$)				
Ertapenem	0.008	0.03	≤0.002 to >32	99.6
Imipenem	0.25	0.25	≤0.03 to >32	99.9
Imipenem + relebactam	0.25/4	0.25/4	≤0.03/4 to 1/4	100
K. pneumoniae ($n = 891$)				
Ertapenem	≤0.125	8	≤0.125 to >8	86
Imipenem	0.25	4	0.06 to >16	88
Imipenem + relebactam	0.25/4	0.25/4	0.06/4 to 2/4	99.3
bla_{KPC}-possessing *K. pneumoniae* ($n = 111$)				
Ertapenem	>8	>8	0.5 to >8	2
Imipenem	16	>16	0.5 to >16	9
Imipenem + relebactam	0.25/4	1/4	0.12/4 to 2/4	97
Enterobacter spp. ($n = 211$)				
Ertapenem	≤0.125	0.25	≤0.125 to >8	93
Imipenem	0.5	1	≤0.03 to >16	90
Imipenem + relebactam	0.25/4	0.5/4	≤0.03/4 to 2/4	99
P. aeruginosa ($n = 490$)				
Imipenem	2	16	≤0.03 to >16	70
Imipenem + relebactam	0.5/4	2/4	≤0.03/4 to >16/4	98
Imipenem-resistant *P. aeruginosa* ($n = 144$)				
Imipenem	8	>16	4 to >16	0
Imipenem + relebactam	1/4	2/4	0.25/4 to >16/4	92
A. baumannii ($n = 158$)				
Imipenem	4	>16	≤0.03 to >16	49
Imipenem + relebactam	2/4	>16/4	≤0.03/4 to >16/4	51
bla_{OXA-23}-possessing *A. baumannii* ($n = 58$)				
Imipenem	>16	>16	≤0.03 to >16	12
Imipenem + relebactam	>16/4	>16/4	≤0.03/4 to >16/4	12

lates. Three *E. aerogenes* isolates and four *E. cloacae* isolates harbored bla_{KPC}. Of these seven isolates, six were not susceptible to imipenem; the MICs ranged from 0.5 to >16 μg/ml. With the addition of relebactam, the MICs ranged from 0.12 to 2 μg/ml, with six isolates being susceptible to imipenem.

Among 490 isolates of *P. aeruginosa*, the imipenem MIC_{50} and MIC_{90} values were 2 and 16 μg/ml, respectively (Table 1). These values decreased to 0.5 and 2 μg/ml, respectively, with the addition of relebactam. Among the 144 isolates that were not susceptible to imipenem, the addition of relebactam resulted in MIC_{50} and MIC_{90} values of 1 and 2 μg/ml, respectively.

Imipenem MICs with and without relebactam were similar among 158 isolates of *A. baumannii* (Table 1). Fifty-eight isolates were found to have $bla_{OXA-23-like}$, two carried $bla_{OXA-24-like}$, and one harbored bla_{KPC}. Eighteen isolates with $bla_{OXA-23-like}$, from eight hospitals, underwent fingerprinting. A total of 10 rep-PCR types were identified, including six belonging to a single clone (data not shown). For the isolates harboring $bla_{OXA-23-like}$, the imipenem MIC_{50} and MIC_{90} values were >16 and >16 μg/ml, respectively, with or without the addition of relebactam. Similarly, the imipenem MICs for the two isolates with $bla_{OXA-24-like}$ did not change with the addition of relebactam. For a single isolate with bla_{KPC}, the imipenem MIC decreased from >16 μg/ml to 4 μg/ml with the addition of relebactam.

Results with previously characterized isolates. Fourteen previously characterized isolates of KPC-producing *K. pneumoniae* were examined (10, 11). In the presence of relebactam, imipenem MICs did not correlate with the expression of *ramA*, *acrB*, or bla_{KPC}. Similarly, there was no correlation among 8 isolates without frameshift mutations in *ompK35*. Ten isolates had expression of *ompK36* greater than the control levels, with imipenem MICs ranging from 2 to >16 μg/ml. With the addition of relebactam, all of the imipenem MICs were 0.25 to 0.5 μg/ml. Four isolates had reduced expression of *ompK36*; the imipenem MICs for those isolates were 4, >16, >16, and >16 μg/ml. With the addition of relebactam, the imipenem MICs decreased to 0.5, 2, 2, and 8 μg/ml, respectively. The latter isolate did not have amplifiable *ompK36*.

Thirty previously characterized isolates of *P. aeruginosa* were analyzed (12); none possessed carbapenemases. Six isolates were wild type regarding *ampC* and *oprD* expression (similar to control). Imipenem MICs ranged from 2 to 4 μg/ml for this group, and all of the isolates had imipenem MICs of 1 μg/ml with the addition of relebactam. Fourteen isolates had reduced *oprD* expression with wild-type *ampC* expression. For these isolates, the imipenem MICs ranged from 1 to >16 μg/ml. With the addition of relebactam, the MICs decreased to 0.25 to 8 μg/ml (average, 1.8 ± 1.9 μg/ml). Ten isolates had reduced *oprD* expression and

upregulated *ampC* expression. The imipenem MICs for these isolates ranged from 2 to >16 μg/ml. With the addition of relebactam, the MICs ranged from 1 to 8 μg/ml (average, 4.6 ± 2.9 μg/ml).

Twenty-eight previously characterized isolates of *A. baumannii* were also included (13). In general, imipenem MICs were unchanged with the addition of relebactam. There was no clear relationship between the expression of *ampC*, $bla_{\text{oxa-51}}$, *adeB*, and *abeM* and the MICs for imipenem with relebactam.

The global spread of carbapenemases in pathogens that are already resistant to other classes of antibiotics has posed a serious therapeutic challenge for clinicians. RPX7009, avibactam, and relebactam are novel β-lactamase inhibitors with activity against primarily class A and class C β-lactamases (3, 4). When combined with imipenem, relebactam has demonstrated dose-dependent synergy against a small number of *Enterobacteriaceae* strains harboring bla_{KPC} (14, 15). In this report, relebactam (at a fixed concentration of 4 μg/ml) restored imipenem susceptibility to 97% of *K. pneumoniae* isolates with bla_{KPC}. When a group of well-characterized isolates were tested, downregulation of *ompK36* appeared to partially offset the protective effect of relebactam. Restoration of imipenem susceptibility was also found for a small number of bla_{KPC}-containing isolates of *E. coli* and *Enterobacter* spp.

In addition, relebactam with imipenem has demonstrated activity against *P. aeruginosa*, including isolates with depressed *oprD* and increased *ampC* expression (14, 15). In our study, the addition of relebactam resulted in approximately 4-fold decreases in the imipenem MIC_{50} and MIC_{90} values, and imipenem susceptibility rates increased from 70% to 98% when relebactam was added. Restoration of imipenem activity was noted for isolates with depressed *oprD* expression, with or without increased *ampC* expression, although the MICs did continue to be higher than those for the wild-type isolates.

The addition of relebactam did not improve the activity of imipenem against *A. baumannii*, however. MICs were unchanged for isolates with overexpression of *ampC* and/or $bla_{\text{OXA-51}}$, suggesting a lack of relebactam activity against these enzymes. Diminished inhibitor activity has been observed against *K. pneumoniae* strains with OXA-48 and absent activity against pathogens harboring metallo-β-lactamases (15). Further development of new antimicrobial agents directed against pathogens harboring these β-lactamases is sorely needed.

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