In Vitro Activity of Imipenem-Relebactam Alone or in Combination with Amikacin or Colistin against *Pseudomonas aeruginosa*

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ABSTRACT   Relebactam is a novel class A/C β-lactamase inhibitor that restores imipenem *in vitro* activity against multidrug-resistant and carbapenem-nonsusceptible *Pseudomonas aeruginosa*. Time-kill analyses were performed to evaluate the potential role of imipenem-relebactam in combination with amikacin or colistin against *P. aeruginosa*. Ten clinical *P. aeruginosa* isolates (9 imipenem nonsusceptible) with imipenem-relebactam MICs ranging from 1/4 to 8/4 μg/ml were included. The isolates had varied susceptibilities to imipenem (1 to 32 μg/ml), amikacin (4 to 128 μg/ml), and colistin (0.5 to 1 μg/ml). Duplicate 24-h time-kill studies were conducted using the average steady-state concentrations (Css_{avg}) observed after the administration of imipenem-relebactam at 500 mg/250 mg every 6 hours (q6h) alone and in combination with the Css_{avg} of 25 mg/kg of body weight/day amikacin and 360 mg/day colistin in humans. Imipenem-relebactam alone resulted in 24-h bacterial densities of -2.93 ± 0.38, -1.67 ± 0.29, $+0.38 \pm 0.96$, and $+0.15 \pm 0.65$ $\log_{10}$ CFU/ml at imipenem-relebactam MICs of 1/4, 2/4, 4/4, and 8/4 μg/ml, respectively. No synergy was demonstrated against the single imipenem-susceptible isolate. Against the imipenem-nonsusceptible isolates ($n = 9$), imipenem-relebactam combined with amikacin resulted in synergy (-2.61 ± 1.50 $\log_{10}$ CFU/ml) against all amikacin-susceptible isolates and in two of three amikacin-intermediate (i.e., MIC, 32 μg/ml) isolates (-2.06 ± 0.19 $\log_{10}$ CFU/ml). Synergy with amikacin was not observed when the amikacin MIC was >32 μg/ml. Imipenem-relebactam combined with colistin demonstrated synergy in eight out of the nine imipenem-resistant isolates (-3.17 ± 1.00 $\log_{10}$ CFU/ml). Against these 10 *P. aeruginosa* isolates, imipenem-relebactam combined with either amikacin or colistin resulted in synergistic activity against the majority of strains. Further studies evaluating combination therapy with imipenem-relebactam are warranted.

KEYWORDS β-lactamase, β-lactamase inhibitor, multidrug, synergy, time-kill

Pseudomonas aeruginosa is an important human bacterial pathogen able to cause serious infections in the lung, bloodstream, wounds, urinary tract, and peritoneal cavity, among other organ systems (1–3). The treatment of infection due to *P. aeruginosa* is complicated by this organism's ability to harbor several resistance mechanisms, including β-lactamase expression, efflux pump upregulation, and outer membrane porin loss, which can render several antimicrobials ineffective (4, 5). Antimicrobial resistance has led to limited treatment options for *P. aeruginosa* infections, resulting in increased patient morbidity and mortality (1, 6).

Historically, carbapenem antibiotics (i.e., meropenem and imipenem) have been reserved for the treatment of multidrug-resistant (MDR) *P. aeruginosa* infections since they often retained activity in the face of resistance to other antipseudomonal β-lactams. The isolation of carbapenem-resistant *P. aeruginosa*, however, has now

become conventional at many hospitals in North America. Recent surveillance studies report *P. aeruginosa* carbapenem nonsusceptibility rates of 20% to 25% and up to 35% in the intensive care unit setting (7–9); furthermore, carbapenem nonsusceptibility generally mirrors the prevalence of MDR isolates. As a result, new treatments for carbapenem-resistant and MDR *P. aeruginosa* are needed (10). Imipenem-relebactam is a carbapenem–β-lactamase inhibitor combination currently in clinical development for the treatment of hospital-acquired bacterial pneumonia (HABP), ventilator-associated bacterial pneumonia (VABP), complicated intra-abdominal infection (cIAI), and complicated urinary tract infection (cUTI) caused by MDR pathogens (ClinicalTrials.gov identifiers NCT02452047 and NCT02493764) (11). The addition of relebactam to imipenem-cilastatin restored its activity in 78% of imipenem-nonsusceptible *P. aeruginosa* strains (8).

Current Infectious Diseases Society of America (IDSA) guidelines recommend empirical combination therapy with two antipseudomonal agents from different classes to treat HABP/VABP due to *P. aeruginosa* bacteria in high-risk patients (3). Dual antipseudomonal therapy is also recommended for HABP/VABP patients with risk factors for MDR Gram-negative pathogens, patients in units where more than 10% of Gram-negative isolates are resistant to an agent being considered for monotherapy, and patients in an intensive care unit where local antimicrobial susceptibility rates are not available (3). Combination antimicrobial therapy is an attractive treatment strategy often considered for the most serious *P. aeruginosa* infections, as it has the potential to broaden empirical coverage, take advantage of potential synergy between the two antibiotics, and prevent the emergence of resistance to one or both of the active agents (12). Despite evidence of synergy exhibited by various antimicrobial combinations *in vitro*, combination therapy has not been strongly associated with improved clinical outcomes, except in the most severe cases of infection (3, 12–14). As imipenem-relebactam is a potent, yet novel, antibiotic, we sought to assess the potential for synergistic interactions with two other antibiotic classes typically reserved for serious MDR *P. aeruginosa* cases, amikacin and colistin (3).

Methods for assessing antimicrobial *in vitro* interactions include disk diffusion tests, checkerboard titrations, time-kill assays, and *in vitro* pharmacodynamic modeling. The optimal methodology, concentration, and interpretation criteria vary widely within these tests; however, time-kill assays remain a simple and effective testing strategy to assess the rate and extent of bacterial kill with antimicrobial agents (15–17). Utilizing time-kill assays, we assessed the synergistic effect of clinical concentrations of imipenem-relebactam in combination with amikacin and colistin against MDR *P. aeruginosa*, including carbapenem-nonsusceptible isolates.

RESULTS

P. aeruginosa susceptibility. The susceptibilities of the 10 selected isolates to imipenem-relebactam, amikacin, colistin, and comparator antibiotics are presented in Table 1. Nine out of the 10 isolates met the definition for MDR and were also nonsusceptible to imipenem and meropenem. Imipenem-relebactam MICs ranged from 1/4 to 8/4 μg/ml. All 10 isolates were susceptible to colistin ($<$2 μg/ml), and amikacin MICs ranged from 4 to 128 μg/ml.

Time-kill analyses. Time-kill curves for imipenem-relebactam, amikacin, and colistin alone and in combination are shown in Fig. 1 and 2. The mean bacterial density of the starting inoculum was 6.55 $\pm$ 0.28 $\log_{10}$ CFU/ml across all isolates examined. Drug-free control experiments resulted in CFU increases of 1.69 $\pm$ 0.37 $\log_{10}$ CFU/ml after 24 h. Imipenem-relebactam alone resulted in 24-h CFU changes of $-$2.93 $\pm$ 0.38, $-$1.67 $\pm$ 0.29, $+$0.38 $\pm$ 0.96, and $+$0.15 $\pm$ 0.65 $\log_{10}$ CFU/ml for isolates with imipenem-relebactam MICs of 1/4, 2/4, 4/4, and 8/4 μg/ml, respectively. At 24 h, amikacin alone resulted in CFU reduction in isolates with an amikacin MIC of $\leq$8 μg/ml ($-$1.52 $\pm$ 0.72 $\log_{10}$ CFU/ml, $n = 3$), while regrowth was observed in remaining isolates with an amikacin MIC of $\geq$16 μg/ml ($+$1.39 $\pm$ 0.53 $\log_{10}$ CFU/ml), relative to the 0-h controls. Colistin alone produced rapid killing over the first 3 to 6 h in all 10

TABLE 1 MICs of imipenem-relebactam, amikacin, colistin, and comparator agents against 10 *P. aeruginosa* isolates

Isolate[a]	MIC (µg/ml) for[b]:										
	AMK	ATM	CAZ	CIP	CST	FEP	IPM	I/R	MEM	TZP	TOB
CAIRD M9-44	4	8	2	0.25	0.5	2	1	1/4	0.5	4	1
CAIRD M8-29	8	32	32	>16	1	16	4	1/4	8	64	1
CDC0508[c]	8	8	16	>16	1	16	16	2/4	8	64	1
CAIRD M3-1	128	16	4	>16	0.5	16	16	2/4	8	256	>64
CAIRD M6-6	16	16	32	>16	1	16	16	4/4	64	16	128
CAIRD M23-3	32	8	64	>16	1	32	32	4/4	16	256	128
CAIRD M1-13	64	>64	>64	>16	1	>64	32	4/4	32	64	>64
CAIRD M3-34	128	16	8	>16	1	16	16	4/4	16	256	>64
CDC0526[d]	32	>64	>64	4	0.5	64	16	8/4	32	32	>64
CAIRD M1-4	32	64	8	>16	0.5	64	32	8/4	>64	128	2

[a]Unless noted otherwise, all isolates are clinical respiratory or blood *P. aeruginosa* isolates collected from the U.S. hospital surveillance study (46).
[b]AMK, amikacin; ATM, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; CST, colistin; FEP, cefepime; IPM, imipenem; I/R, imipenem-relebactam; MEM, meropenem; TZP, piperacillin-tazobactam; TOB, tobramycin.
[c]Obtained from the FDA-CDC Antimicrobial Resistance Isolate Bank (Atlanta, GA). Isolate CDC0508 has chromosomally encoded bla_{PDC-35} and bla_{PER-1} β-lactamases.
[d]Obtained from the FDA-CDC Antimicrobial Resistance Isolate Bank. Isolate CDC0526 has a chromosomally encoded bla_{PDC-19} β-lactamase.

isolates, followed by growth at 24 h to a mean bacterial density (6.54 ± 0.77 $\log_{10}$ CFU/ml) on par with the 0-h starting inoculum.

Table 2 shows the antimicrobial interactions (i.e., synergy, additivity, indifference, and antagonism) observed with imipenem-relebactam in combination with amikacin or colistin against all evaluated isolates. The combination of imipenem-relebactam and amikacin was rapidly bactericidal against all isolates at 6 h. By 24 h, additivity or synergy was observed in 7 isolates. The subgroup of isolates that did not demonstrate synergy were highly resistant to amikacin (MIC, ≥64 µg/ml; $n = 3$) or susceptible to imipenem
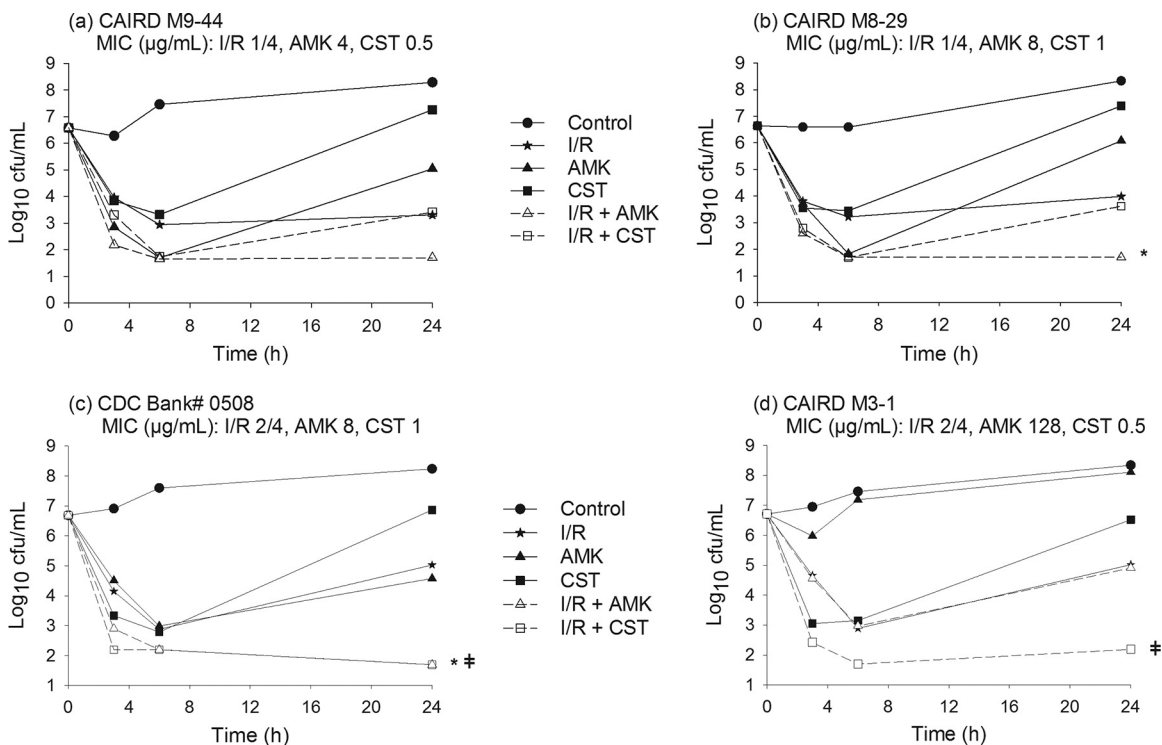
FIG 1 Time-kill experiments displaying the activity of imipenem-relebactam alone and in combination with amikacin or colistin against *P. aeruginosa* isolates with imipenem-relebactam MICs of 1/4 µg/ml and 2/4 µg/ml. I/R, imipenem-relebactam; AMK, amikacin; CST, colistin; *, synergy with I/R plus AMK; ‡, synergy with I/R plus CST.
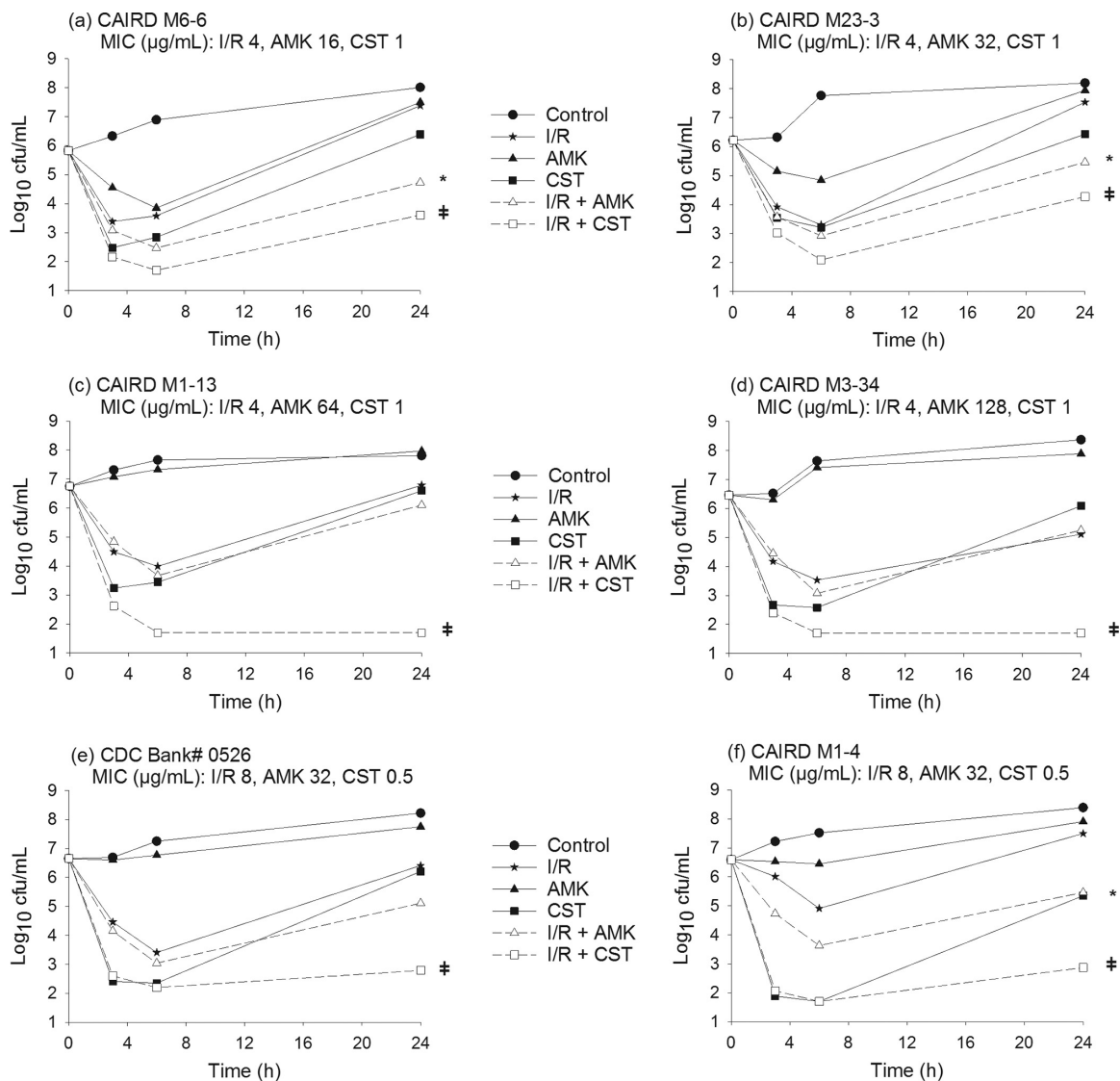
FIG 2 Time-kill experiments displaying the activity of imipenem-relebactam alone and in combination with amikacin or colistin against *P. aeruginosa* isolates with imipenem-relebactam MICs of 4/4 μg/ml and 8/4 μg/ml. I/R, imipenem-relebactam; AMK, amikacin; CST, colistin; *, synergy with I/R plus AMK; ‡, synergy with I/R plus CST.

(MIC, <2 μg/ml; *n* = 1). Of note, the combination of imipenem-relebactam and amikacin was additive or synergistic in the 3 isolates with intermediate susceptibility to amikacin (MIC, 32 μg/ml) evaluated in this study.

The combination of imipenem-relebactam and colistin was rapidly bactericidal against all isolates at 6 h and met the definition of synergy at 24 h in 8 of the 10 isolates. The 2 isolates that showed bacterial indifference with this combination had the lowest imipenem-relebactam MICs (i.e., 1/4 μg/ml); thus, the addition of colistin did not result in further CFU reduction beyond that with imipenem-relebactam alone.

DISCUSSION

Multidrug resistance in *P. aeruginosa* is a growing public health problem and is challenging to treat. Combination therapy is routinely used in clinical practice to broaden the antimicrobial spectrum and decrease the risk of initial inappropriate therapy, although its potential for antimicrobial synergy and delaying or preventing antimicrobial resistance remain appealing theoretical indications (12). In this study, imipenem-relebactam-based combination therapy resulted in bactericidal and syner-

TABLE 2 Antimicrobial interactions observed with imipenem-relebactam in combination with amikacin or colistin against 10 *P. aeruginosa* isolates at 24 h

| | Interaction for combination[a]: | |
Isolate	I/R plus AMK	I/R plus CST
CAIRD M9-44	Additivity	Indifference
CAIRD M8-29	Synergy	Indifference
CDC0508	Synergy	Synergy
CAIRD M3-1	Indifference	Synergy
CAIRD M6-6	Synergy	Synergy
CAIRD M23-3	Synergy	Synergy
CAIRD M1-13	Indifference	Synergy
CAIRD M3-34	Indifference	Synergy
CDC0526	Additivity	Synergy
CAIRD M1-4	Synergy	Synergy

[a]AMK, amikacin; CST, colistin; I/R, imipenem-relebactam.

gistic effects against the majority of the imipenem-nonsusceptible *P. aeruginosa* isolates evaluated.

Relebactam is an investigational non-β-lactam–β-lactamase inhibitor in clinical development. Paired with imipenem, relebactam has potent *in vitro* activity against class A β-lactamases, including KPC-type carbapenemases, and class C β-lactamases (11, 18). In a recent intensive care unit (ICU)-based surveillance study with 538 *P. aeruginosa* isolates, the respective susceptibility rates of meropenem, imipenem, and imipenem-relebactam were 72.7%, 67.1%, and 91.5% (9). The carbapenem-nonsusceptible and MDR *P. aeruginosa* isolates utilized in this study are representative of isolates that may be encountered in a critically ill population and where antimicrobial agents such as imipenem-relebactam would be considered for treatment.

Time-kill assays are a reliable *in vitro* test for the assessment of antimicrobial synergy. Compared with the Etest and checkerboard methodology, time-kill assays examine the magnitude of bacterial killing over time (17). Despite their wide use, debate continues over the optimal antimicrobial concentration to be assessed in time-kill assays; thus, investigators utilize a variety of approaches, including fractions of the isolate's MIC (16, 19). Consequently, the amikacin and colistin concentrations utilized in this study were based on average steady-state concentrations (Css_{avg}) achieved in critically ill patients when administered at clinically relevant doses, while imipenem-relebactam, due to its investigational status, was employed at clinically achievable steady-state concentrations based on healthy volunteer data. In contrast to using an MIC fraction, peak, or trough concentration, the free Css_{avg} methodology reflects the average clinical antimicrobial concentration available to interact with bacteria over 24 h. It is worth noting that critically ill patients tend to have high intra- and interpatient variability in drug pharmacokinetics; thus, antimicrobial steady-state, peak, or trough concentrations obtained from the literature for utilization in *in vitro* studies may vary. The MIC fraction methodology is also prone to similar challenges given the $\pm$1 2-fold concentration variation associated with MIC determination (20). Importantly, the colistin and amikacin average steady-state concentrations utilized in this study are similar to reported concentrations from other critically ill cohorts (21, 22).

The magnitude of bacterial killing with imipenem-relebactam alone across the range of imipenem-relebactam MICs was consistent with the imipenem-relebactam free Css_{avg} tested *in vitro*. Bacterial reductions of >2 log and >1 log were observed at imipenem-relebactam MICs of 1/4 μg/ml and 2/4 μg/ml, respectively, while bacteriostatic effects were observed at MICs of $\geq$4/4 μg/ml relative to the 0-h controls. Given enhanced imipenem-relebactam activity against *P. aeruginosa* (8, 10, 18), we included isolates with imipenem-relebactam MICs above the current imipenem breakpoint to assess an MIC distribution that may encompass a potential imipenem-relebactam MIC breakpoint. Notably, *P. aeruginosa* isolates with imipenem-relebactam MICs of $\geq$4 μg/ml represent less than 6% of the MIC distribution (8).

Imipenem-relebactam-based combination therapy resulted in synergy against the

majority of the isolates evaluated in this study; however, the combination of imipenem-relebactam and amikacin proved to be ineffective against amikacin-resistant *P. aeruginosa* isolates. The underlying mechanism of β-lactam–aminoglycoside synergy is thought to be mediated by both agents. The inhibition of bacterial cell wall synthesis by β-lactams facilitates the entry of aminoglycosides into the bacterial cell (12, 23), while the ionic binding of positively charged aminoglycoside molecules to negatively charged bacterial cell surface components, including outer membrane proteins, results in increased permeability of aminoglycosides and other antimicrobial agents (24, 25). Irrespective of the imipenem-relebactam MIC, enhanced bacterial killing (i.e., synergy or additivity) with the combination of imipenem-relebactam plus amikacin was diminished as the MIC of the aminoglycoside increased beyond an amikacin MIC of $\geq$64 μg/ml.

Numerous *in vitro* studies have examined aminoglycosides in combination with β-lactams against *P. aeruginosa*, albeit with a variety of drug concentrations (26–31). In a time-kill study by Yadav et al. (31), imipenem combined with either tobramycin or amikacin demonstrated synergy and suppression of resistance against 3 imipenem-resistant *P. aeruginosa* isolates. Notably, the imipenem concentrations studied included the highest clinically achievable steady-state plasma concentrations possible after the administration of imipenem doses of 4 g/day (31). Though FDA approved, imipenem doses of 4 g/day are not routinely utilized in clinical practice, given a trend toward an increased risk of seizure with doses of >2 g/day (32, 33). The high imipenem concentration utilized in the investigation by Yadav and colleagues (31) may explain the synergistic killing observed among aminoglycoside-resistant *P. aeruginosa* strains, in contrast to our observations.

Colistin alone, utilized at a steady-state concentration achieved in critically ill patients, was initially bactericidal, but significant regrowth occurred at 24 h in 50% of the isolates, despite *in vitro* colistin susceptibility. Regrowth of colistin-susceptible *P. aeruginosa* (i.e., colistin heteroresistance) is well described in the literature (34–37). To mitigate the potential for heteroresistance with colistin monotherapy, combination therapy against *P. aeruginosa* has been suggested as a possible means by which to increase antimicrobial activity and mitigate resistance development (35, 37). Indeed, a meta-analysis of data (*n* = 136 *P. aeruginosa* isolates) from published time-kill studies with either colistin or polymyxin in combination with a carbapenem showed substantial synergy rates (50% [95% confidence interval {CI}, 30% to 69%]) and evidence of resistance suppression *in vitro* (38). In this study, combining the novel imipenem-relebactam with colistin resulted in synergy in 8 of 9 imipenem-resistant *P. aeruginosa* isolates evaluated.

In summary, we assessed average steady-state concentrations of imipenem-relebactam in combination with amikacin and colistin against MDR and carbapenem-nonsusceptible *P. aeruginosa* isolates using *in vitro* time-kill methodology. These data demonstrate the bactericidal and synergistic antimicrobial effects of an imipenem-relebactam-based combination therapy with no evidence of antagonism. These data support the role of imipenem-relebactam as part of a promising therapeutic strategy for the treatment of *P. aeruginosa* infections. Further *in vitro* studies and pharmacokinetic/pharmacodynamic analyses of these combinations are warranted against challenging-to-treat *P. aeruginosa* strains.

MATERIALS AND METHODS

Bacterial isolates. Ten clinical *P. aeruginosa* isolates were selected for this study. Two isolates were obtained from the FDA-CDC Antimicrobial Resistance Isolate Bank (Atlanta, GA), and the remaining 8 isolates were from the Center for Anti-Infective Research and Development (CAIRD) isolate repository of clinical strains collected during a recent U.S.-based surveillance study between July 2017 and June 2018. Isolates were chosen to display a range of imipenem-relebactam MICs of 1/4, 2/4, 4/4, and 8/4 μg/ml. This range of imipenem-relebactam MICs allowed an assessment of isolates at or within 2 dilutions of the current Clinical and Laboratory Standards Institute (CLSI) breakpoint (2 μg/ml) for imipenem against *P. aeruginosa* (39). Additionally, isolates were selected for a range of amikacin MICs from susceptible to resistant. Ultimately, 9 imipenem-nonsusceptible isolates were included; in addition, 1 imipenem-susceptible isolate was included to determine if synergy is observed regardless of imipenem susceptibility. All isolates were maintained in skim milk (BD Biosciences, Sparks, MD) at −80°C. Each isolate was

subcultured twice on Trypticase soy agar with 5% sheep blood (BD Biosciences) and grown for 18 to 20 h at 37°C under 5% CO_2 prior to use in the experiments.

Susceptibility testing. Susceptibilities to amikacin, colistin, imipenem, and imipenem-relebactam, as well as commonly utilized antipseudomonal agents, were determined for all isolates using the broth microdilution methodology, as outlined by the CLSI (39). Antibiotics were obtained as laboratory-grade powders from their respective manufacturers. MIC testing for imipenem-relebactam used a fixed relebactam concentration of 4 μg/ml in combination with doubling dilutions of imipenem. MIC values were obtained in triplicate, and the modal MIC was reported. *P. aeruginosa* ATCC 27853 was used as a quality control strain, as defined by the CLSI (39). In addition, to assess the appropriate concentration of relebactam in microdilution trays, *P. aeruginosa* CDC0516, harboring a KPC-2 enzyme, was used as an additional quality control (range, 0.5/4 to 2/4 μg/ml).

Simulated drug exposures. Time-kill studies were performed using the average steady-state concentration (Css_{avg}) of imipenem and relebactam alone and in combination with the Css_{avg} of amikacin and colistin. Imipenem and relebactam plasma clearance (in liters per hour) following the administration of a single dose of imipenem-cilastatin (500 mg) with relebactam (500 mg) to healthy volunteers in a phase 1 study was used to calculate the free area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) of imipenem and relebactam achieved with the administration of 500/250 mg imipenem-relebactam every 6 h (40). After applying a protein binding factor of 20% for imipenem and relebactam (41), the simulated target imipenem and relebactam free Css_{avg} over a 24-h period were 5.9 μg/ml and 4.2 μg/ml, respectively. Amikacin was simulated to achieve a free Css_{avg} of 14.5 μg/ml consistent with that achieved with a 25-mg/kg/day dose in a 70-kg critically ill patient with severe sepsis (42). Colistin was simulated as a continuous infusion to achieve the mean free Css_{avg} (0.73 μg/ml) for critically ill adult patients receiving the currently recommended European Medicines Agency (EMA) dosing regimen of 360 mg daily of colistin base activity (43).

Time-kill studies. Time-kill analyses were performed on each of the 10 *P. aeruginosa* isolates. Mueller-Hinton broth (Becton, Dickinson and Company, Sparks, MD) was inoculated with the bacterial suspension to a final suspension of approximately 10^6 CFU/ml. Duplicate time-kill experiments with imipenem-relebactam alone, amikacin alone, colistin alone, and imipenem-relebactam in combination with amikacin and colistin were conducted. Control experiments without any active agents were conducted simultaneously with the time-kill studies. The final volume for each bacterium-drug concentration was 10 ml, and the suspensions were incubated in a shaking water bath at 37°C. Samples were taken from each experiment at 0, 3, 6, and 24 h, diluted, and incubated for 18 to 24 h on blood agar plates to quantify the mean bacterial densities. The minimal accurately countable number of CFU per milliliter was determined to be 5×10^{-1} CFU/ml. Bactericidal activity of single antibiotics or combinations was defined as a $\geq$3-$\log_{10}$ reduction in CFU/ml of the initial inoculum after 24 h of incubation. Synergy was defined as a $\geq$2-$\log_{10}$ decrease in CFU/ml between the antibiotic combination and its most active constituent after 24 h. Additivity was defined as a 1- to $<$2-$\log_{10}$ decrease in CFU/ml between the antibiotic combination and its most active constituent after 24 h, while indifference was defined by a $<$1-$\log_{10}$ CFU/ml change between the antibiotic combination and its most active constituent. Antagonism ($\geq$2-$\log_{10}$ increase in CFU/ml at 24 h with the combination compared with that by the most active drug alone) was also noted (44, 45).

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