Novel Approach To Optimize Synergistic Carbapenem-Aminoglycoside Combinations against Carbapenem-Resistant Acinetobacter baumannii

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Acinetobacter baumannii is among the most dangerous pathogens and emergence of resistance is highly problematic. Our objective was to identify and rationally optimize β-lactam-plus-aminoglycoside combinations via novel mechanism-based modeling that synergistically kill and prevent resistance of carbapenem-resistant A. baumannii. We studied combinations of 10 β-lactams and three aminoglycosides against four A. baumannii strains, including two imipenem-intermediate (MIC, 4 mg/liter) and one imipenem-resistant (MIC, 32 mg/liter) clinical isolate, using high-inoculum static-concentration time-kill studies. We present the first application of mechanism-based modeling for killing and resistance of A. baumannii using Monte Carlo simulations of human pharmacokinetics to rationally optimize combination dosage regimens for immunocompromised, critically ill patients. All monotherapies achieved limited killing (<2.3 log10) of A. baumannii ATCC 19606 followed by extensive regrowth for aminoglycosides. Against this strain, imipenem-plus-aminoglycoside combinations yielded more rapid and extensive killing than other β-lactam-plus-aminoglycoside combinations. Imipenem at 8 mg/liter combined with an aminoglycoside yielded synergistic killing (>5 log10) and prevented regrowth of all four strains. Modeling demonstrated that imipenem likely killed the aminoglycoside-resistant population and vice versa and that aminoglycosides enhanced the target site penetration of imipenem. Against carbapenem-resistant A. baumannii (MIC, 32 mg/liter), optimized combination regimens (imipenem at 4 g/day as a continuous infusion plus tobramycin at 7 mg/kg of body weight every 24 h) were predicted to achieve >5 log10 killing without regrowth in 98.2% of patients. Bacterial killing and suppression of regrowth were best achieved for combination regimens with unbound imipenem steady-state concentrations of at least 8 mg/liter. Imipenem-plus-aminoglycoside combination regimens are highly promising and warrant further evaluation.

Antimicrobial resistance in Gram-negative bacteria is one of the three greatest threats to human health (1–3). Acinetobacter baumannii is one of the three most challenging Gram-negative pathogens, especially in intensive care units. In approximately 14,000 critically ill patients, A. baumannii infections were highly associated (P < 0.001) with increased mortality (4). A. baumannii often causes bloodstream, respiratory tract (including ventilator-associated pneumonia), and wound infections (including burns and combat wounds); these are associated with high morbidity (1, 2, 5) and up to 87% mortality (6). Multidrug-resistant A. baumannii strains have caused major outbreaks in the United States and worldwide (7, 8).

In the past, β-lactams and aminoglycosides were successfully used to treat susceptible A. baumannii (9), but unfortunately, strains have emerged that are resistant to virtually all antibiotics in monotherapy (10, 11). While carbapenems were hitherto considered the treatment of choice against severe A. baumannii infections, carbapenem-resistant A. baumannii isolates are rapidly increasing (11). Aminoglycoside monotherapy can cause significant killing of A. baumannii but is followed by rapid and extensive resistance emergence in vitro and in patients (12–14). The high rates of A. baumannii resistance highlight the urgent need for alternative treatment options, such as rationally optimized combination therapies.

A small number of in vitro and animal infection model studies assessed β-lactam-plus-aminoglycoside combinations and usually studied only one β-lactam and/or one aminoglycoside (12, 15, 16). We are aware neither of a systematic evaluation of monotherapies and combinations for a series of β-lactams and aminoglycosides in A. baumannii nor of any study which used time course modeling to optimize monotherapies or combinations against A. baumannii.

β-Lactam antibiotics are widely used and very safe, and clinicians worldwide are well trained in the safe use of aminoglycosides (17). Aminoglycoside and β-lactam antibiotics have different mechanisms of action and resistance; there is no efflux pump which affects both of these antibiotic classes in A. baumannii (18). This suggests that β-lactams may kill aminoglycoside-resistant bacteria and vice versa (subpopulation synergy [19, 20]). Additionally, disruption of the outer membrane by an aminoglycoside may enhance the target site penetration of β-lactams, since the outer membrane of A. baumannii is approximately 2- to 7-fold thinner.

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less permeable than that of Pseudomonas aeruginosa and approximately 50-fold less permeable than that of Escherichia coli (21, 22).

The first objective of this study was to identify synergistic bacterial killing and prevention of resistance for combinations of a β-lactam with an aminoglycoside against A. baumannii. The second objective was to quantify the extent, time course, and potential mechanisms of synergy via novel mechanism-based modeling of antibiotic combinations. Our final objective was to rationally optimize combination dosage regimens for immunocompromised, critically ill patients with bacteremia caused by carbapenem-resistant A. baumannii. These Monte Carlo simulations utilized novel mechanism-based models for bacterial killing and resistance and human population pharmacokinetic models to prospectively optimize combination dosage regimens for future studies in animal infection models and ultimately humans.

(Thus, this work was presented as an oral presentation [23] at the 53rd Interscience Conference on Antimicrob Agents and Chemotherapy, Denver, CO, 10 to 13 September 2013; as a poster presentation at the 2014 Annual Scientific Meeting of the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists, Melbourne, Australia, 7 to 11 December 2014; and as an oral presentation at the 17th annual meeting of the Population Approach Group in Australia and New Zealand, Melbourne, Australia, 28 to 30 January 2015.)

MATERIALS AND METHODS

Bacterial isolates, media, and susceptibility testing. The A. baumannii strain ATCC 19606 and three clinical A. baumannii isolates (FADDI-AB014, FADDI-AB016, and FADDI-AB034) from the collection at Monash University were used for all experiments. All susceptibility testing and static-concentration time-kill experiments (SCTK) were performed in cation-adjusted Mueller-Hinton broth (CAMHB; BBL, BD, SParks, MD). Viable counting was conducted on cation-adjusted Mueller-Hinton II agar (CAMHA; Medium Preparation Unit, The University of Melbourne). Stock solutions of imipenem (Merck Sharp & Dohme Pty, New South Wales, Australia), isepamicin (Waterstone Technology, LLC, Carmel, IN), amikacin (Sigma-Aldrich, St. Louis, MO), and tobramycin (AK Scientific, Inc., Union City, CA) were prepared in sterile distilled water and filter sterilized with a Millex-GV 0.22-µm polyvinylidene difluoride (PVDF) syringe filter (Merck Millipore Ltd., Cork, Ireland). The MICs were determined in triplicate according to the Clinical and Laboratory Standards Institute guidelines (24), and EUCAST breakpoints were used to define carbapenem resistance (25).

Static-concentration time-kill experiments. All A. baumannii strains were evaluated via in vitro SCTK experiments over 2 days. We studied monotherapy and combination therapies of 10 β-lactams and three aminoglycosides in A. baumannii ATCC 19606. Subsequent SCTK experiments assessed imipenem plus two aminoglycosides (tobramycin and isepamicin) in three imipenem-intermediate or imipenem-resistant clinical isolates. The antibiotic concentrations studied included the highest clinically achievable average unbound plasma concentrations at steady state. A tobramycin concentration of 4 mg/liter and isepamicin concentrations of 8 to 16 mg/liter represent the average unbound plasma concentrations in humans over a 24-h dosing interval for tobramycin at 7 mg/kg of body weight and 15 or 25 mg/kg isepamicin given once daily (17, 26). Additional experimental arms with 12 mg/liter tobramycin and 64 mg/liter isepamicin represent the average free plasma concentration of aminoglycosides during the first 6 h. For the highest clinically approved imipenem doses of 4 g per day, our Monte Carlo simulations predicted the unbound average steady-state concentrations to range from 7.61 to 22.6 mg/liter in adult critically ill patients (27).

The SCTK experiments were performed at a high initial inoculum using previously described methods (20, 28, 29). Serial broth samples were taken before dosing and at multiple time points over 2 days. At 24 h, the bacterial suspension was centrifuged, supernatant was removed, and bacteria were resuspended in fresh, prewarmed broth with the targeted antibiotic concentration. To further offset the thermal degradation of carbapenems, a carbapenem amount of 50% of the original dose was supplemented at 6 and 30 h. Our liquid chromatography-tandem mass spectrometry (LC-MS/MS) data (30) showed approximately 54% degradation of imipenem at 1 and 100 mg/liter in CAMHB after 24 h of incubation at 35°C. This supplementation procedure ensured that the targeted antibiotic concentration was achieved every 24 h and that the average carbapenem concentration was maintained throughout the experiment. Bacteria in all broth samples were washed twice by centrifugation and resuspension in fresh sterile saline. Viable counts were determined by manual plating of 100 µl of an undiluted or appropriately diluted suspension in saline onto CAMHA plates (20, 28, 29).

Mechanism-based modeling of antibiotic combinations. To characterize the extent, time course, and potential mechanisms of synergy for a β-lactam plus an aminoglycoside, mechanism-based pharmacodynamic (PD) modeling was performed. These mechanism-based models of bacterial killing and resistance are ideally suited to be used with human population pharmacokinetic models to predict and rationally optimize combination dosage regimens in humans.

Life cycle growth model. A life cycle growth model (Fig. 1A) was used to describe the underlying biology of bacterial replication (31, 32). Bacterial replication for each population was defined via two states. Bacteria which are growing and preparing for replication reside in state 1, and bacteria which are immediately before replication reside in state 2. The transition from state 1 to state 2 was governed by a first-order growth rate constant (k12) which determines the mean generation time (MGT), since replication was assumed to be fast (k12 was fixed at 50 h–1) (31). We considered models with a longer mean generation time for the resistant populations.

Antibiotic combinations. The model for the combinations of imipenem and an aminoglycoside contained three pre-existing populations; including a double-susceptible (SS), an imipenem-resistant aminoglycoside-intermediate (RI), and an imipenem-intermediate aminoglycoside-resistant (IR) bacterial population (Fig. 1B). As each population was described by two states (i.e., compartments) in the life cycle growth models, the full model contained six compartments. The total concentration of all viable bacteria (CFUall) was described as follows:

$$CFU_{all} = CFU_{SS1} + CFU_{SS2} + CFU_{RI1} + CFU_{RI2} + CFU_{IR1} + CFU_{IR2}$$

(1)

Each CFUxin term represents the concentration of viable bacteria for population NN in state x. The rate of bacterial growth was assumed to be inhibited at high concentrations of hypothetical signal molecules (Csig) as described by the term lnhi12 (31).

$$lnhi12 = \left( I_{max,sig12} \cdot C_{sig} \right) \left( C_{sig} + IC_{50,sig} \right)$$

(2)

I_{max,sig12} is the maximum extent of inhibition of the rate of replication at high Csig and IC_{50,sig} is the signal molecule concentration associated with 50% of I_{max,sig12}.

The maximum rate of killing by imipenem or an aminoglycoside in the present data set was higher than the maximum rate of growth. Therefore, we specified killing by either antibiotic via a direct killing process described by a Hill function. The differential equation for the concentration of bacteria belonging to the double-susceptible population in state 1 (CFU_{SS1}) comprised killing by imipenem and the aminoglycoside (initial conditions, model parameters, and variables described below):
where $C_{IPM}$ is the imipenem concentration and $C_{AGS}$ the aminoglycoside concentration (i.e., tobramycin, isepamicin, or amikacin) in broth. $CFU_{SS2}$ is the bacterial concentration in state 2 of the double-susceptible population. The plateau factor (PLAT) represents the probability of successful replication and is defined as \( 1 / \left[ \frac{CFU_{all}}{CFU_{max}} \right] \), with $CFU_{max}$ being the maximum population size. The factor 2 in equation 3 represents the doubling of bacteria during replication. When $CFU_{all}$ is much smaller than $CFU_{max}$, PLAT approaches 100%. As the $CFU_{all}$ approaches $CFU_{max}$, the probability of successful replication approaches 50%, yielding stasis of the bacterial population (i.e., on average, one of two doublings is successful). The maximum killing rate constants ($K_{max}$), the associated concentrations ($KC_{50}$) causing 50% of $K_{max}$, and the Hill coefficients for the respective antibiotic and population are illustrated in Fig. 1B. We assumed that killing by imipenem and the aminoglycoside affected both states and thus included the same killing terms for states 1 and 2. This yields the differential equation for state 2 of the double-susceptible population ($CFU_{SS2}$):

\[
\frac{d(CFU_{SS2})}{dt} = -k_{21} \cdot CFU_{SS2} + k_{12 ss} \cdot (1 - Inh_{k12}) \cdot CFU_{SS1} - \left( \frac{K_{max,IPM} \cdot C_{IPM} \cdot Hill_{IPM}}{C_{IPM} \cdot Hill_{IPM} + KC_{50,SS,IPM} \cdot Hill_{IPM}} + \frac{K_{max,SS,AGS} \cdot C_{AGS} \cdot Hill_{AGS}}{C_{AGS} \cdot Hill_{AGS} + KC_{50,SS,AGS} \cdot Hill_{AGS}} \right) \cdot CFU_{SS2}
\]  

(3)

The differential equations for the two resistant populations (i.e., IR and RI) contained the same terms as those for $CFU_{SS1}$ and $CFU_{SS2}$ but contained different estimates for $K_{max}$, $KC_{50}$, and $k_{12}$ compared to the double-susceptible population.

Mechanism-based modeling of synergy. We considered and evaluated subpopulation synergy (i.e., antibiotic A killing the bacteria resistant to antibiotic B and vice versa) and mechanistic synergy (i.e., antibiotic A enhancing the killing by antibiotic B of one or multiple bacterial populations) as previously described (20). Mechanistic synergy was implemented by assuming that the aminoglycoside could enhance the target site penetration of imipenem due to disruption of the outer membrane (33, 34). This was implemented in the model by estimating a lower $KC_{50,IPM}$ in...
the presence of a certain aminoglycoside concentration (e.g., \( \geq 4 \) mg/liter tobramycin). This was implemented in the model code via an IF condition which used the KC_{50,IPM} for combination therapy if the aminoglycoside concentration was at least 4 mg/liter and the KC_{50,IPM} for monotherapy for lower aminoglycoside concentrations.

**Initial conditions.** We estimated the total inoculum (log CFU\(_0\)) and the log\(_{10}\) mutation frequency for each of the two resistant populations. The initial condition of the double-susceptible population was calculated as the difference between CFU\(_0\) and the initial conditions of the two less-susceptible populations. All bacteria were initialized in state 1, and the initial conditions for CFU_{SS2,CFU_{R2}} and CFU_{R2} were set at 0 (31).

**Observation model.** The log\(_{10}\) viable counts were fitted using an additive residual error model on a log\(_{10}\) scale. For observations below 100 CFU/ml (equivalent to fewer than 10 colonies per plate), the number of colonies per plate was directly fitted using a previously developed residual error model (28). Viable counts below the limit of counting (i.e., below 1 log\(_{10}\) CFU/ml) and model-predicted viable counts less than 0 log\(_{10}\) CFU/ml were plotted as 0.

**Estimation.** All PD model parameters were simultaneously estimated using all viable-count data from the respective strain via the importance sampling algorithm (pmethod = 4) in parallelized S-ADAPT (version 1.57). The analysis was facilitated by the SADAPT-TRAN tool (35, 36). The between-curve variability of the PD parameters was fixed to a coefficient of variation of 10% during the end of the estimation (28). Competing models were assessed by the objective function (\(-1\times\) log likelihood), plausibility of the parameter estimates, standard diagnostic plots, and visual predictive checks (37, 38).

**Monte Carlo simulations.** We simulated 10,000 adult critically ill patients with normal renal function for each dosage regimen via Monte Carlo simulations using Berkeley Madonna (version 8.3.18). These patients completely lacked any effect of the immune system and were assumed to have bacteremia caused by the most difficult-to-treat, carbapenem-resistant isolate, FADDI-AB034. This isolate had an imipenem MIC of 32 mg/liter, which represents the 97th percentile of the MIC distribution for A. baumannii by EUCAST (http://mic.euCAST.org/Eucast2/), last accessed on 23 September 2014.

For our Monte Carlo simulations, we combined the mechanism-based combination PD model with published population pharmacokinetic (PK) models of tobramycin and imipenem in critically ill patients (26, 27). These Monte Carlo simulations accounted for between-patient variability in the PK of each antibiotic and the between-curve variability of the population PD model parameters. The population PK of imipenem and tobramycin was described by two-compartment, linear models for imipenem and tobramycin (26, 27). For imipenem, we used the reported median PK parameter estimates to represent the population means; the coefficients of variation of these parameters were calculated as the reported standard deviation (SD)/mean (27). For tobramycin, the population means and variances of the PK parameters reported for the final model by Conil et al. were used (26). We predicted the unbound plasma concentrations and viable-count profiles for imipenem and tobramycin using the combined PK/PD model (26, 27).

The simulated regimens included imipenem given as a 1-h infusion of 1 g every 6 or 8 h and imipenem given as a 1-g loading dose (1-h infusion) followed by a continuous infusion of 3 or 4 g per day. Tobramycin was simulated with a dose of 5 or 7 mg/kg given as a 0.5-h infusion every 24 h. Successful therapy was defined by a simulated viable count of the total population of 10\(^{8}\) CFU/ml or less at 168 h. The choice for the cutoff value had limited impact on our results, as the vast majority of simulated viable counts were either well above 10\(^{8}\) CFU/ml or below 10\(^{6}\) CFU/ml at 168 h.

The success rate (\(P_{\text{success}}\)) was calculated as the fraction of the 10,000 simulated patients who attained this target. The failure rate (i.e., probability of patients failing with extensive regrowth of resistant bacteria) was calculated as 1 - \(P_{\text{success}}\).
The imipenem-resistant population displayed stasis or was only slowly killed by imipenem monotherapy, and the aminoglycoside-resistant population caused regrowth during aminoglycoside monotherapy (results not shown).

Subpopulation synergy. Against all four strains, imipenem killed the aminoglycoside-resistant population and aminoglycosides killed the imipenem-resistant population (i.e., subpopulation synergy was present). The imipenem-resistant population...
slowly replicated and required high imipenem concentrations to be killed. The imipenem concentration resulting in half-maximal killing was considerably higher for the imipenem-resistant population (KC50,RI,IPM) than the susceptible population (KC50,SS,IPM) (Table 3). Also, higher aminoglycoside concentrations resulting in half-maximal killing by the aminoglycoside were required for the imipenem-resistant population (KC50,RI,ISE/TOB/AMK) than for the susceptible population (KC50,SS,ISE/TOB/AMK) (Table 3). The aminoglycoside-resistant populations of isolates FADDI-AB016 and FADDI-AB014 were less susceptible to imipenem, as shown by the higher imipenem concentration yielding half-maximal killing of the aminoglycoside-resistant population (KC50,IR,IPM) (Table 3) compared to the susceptible population (KC50,SS,IPM). For these two isolates, subpopulation synergy alone was not sufficient to describe the extensive and synergistic killing by the combinations. Population predictions for the treatment arms containing 4 mg/liter aminoglycoside were considerably better for models with mechanistic synergy than for models without this feature. A thorough modeling analysis identified tobramycin enhancing the

\[
\text{TABLE 1: Log}_{10} \text{ change in viable counts compared to 0-h counts for } A. \text{ baumannii ATCC 19606 in static-concentration time-kill studies at an initial inoculum of } 10^{7.8} \text{ CFU/ml}^a
\]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1.5 h</th>
<th>4.0 h</th>
<th>9.25 h</th>
<th>24 h</th>
<th>50 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth control</td>
<td>0.35</td>
<td>0.64</td>
<td>0.98</td>
<td>0.95</td>
<td>1.07</td>
</tr>
<tr>
<td>Tobramycin (4 mg/liter)</td>
<td>-0.04</td>
<td>-1.38</td>
<td>-1.07</td>
<td>-0.03</td>
<td>-0.13</td>
</tr>
<tr>
<td>Amikacin (4 mg/liter)</td>
<td>0.12</td>
<td>0.10</td>
<td>0.32</td>
<td>0.83</td>
<td>0.04</td>
</tr>
<tr>
<td>Imipenem (1 mg/liter)</td>
<td>-0.28</td>
<td>-1.26</td>
<td>-2.15</td>
<td>-0.08</td>
<td>-0.06</td>
</tr>
<tr>
<td>Imipenem (8 mg/liter)</td>
<td>-0.90</td>
<td>-2.33</td>
<td>-2.51</td>
<td>-2.22</td>
<td>-2.29</td>
</tr>
<tr>
<td>Meropenem (4 mg/liter)</td>
<td>-0.10</td>
<td>-1.43</td>
<td>-2.15</td>
<td>-2.26</td>
<td>-1.88</td>
</tr>
<tr>
<td>Ceftazidime (32 mg/liter)</td>
<td>0.15</td>
<td>-0.16</td>
<td>-0.96</td>
<td>-1.81</td>
<td>-1.91</td>
</tr>
<tr>
<td>Cefsolodin (32 mg/liter)</td>
<td>-0.02</td>
<td>0.08</td>
<td>0.07</td>
<td>0.63</td>
<td>0.76</td>
</tr>
<tr>
<td>Cefepime (32 mg/liter)</td>
<td>-0.02</td>
<td>-0.42</td>
<td>-0.91</td>
<td>0.02</td>
<td>-0.76</td>
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<tr>
<td>Aztreonam (32 mg/liter)</td>
<td>-0.01</td>
<td>-0.05</td>
<td>-0.06</td>
<td>0.21</td>
<td>-0.11</td>
</tr>
<tr>
<td>Carbencillin (64 mg/liter)</td>
<td>0.15</td>
<td>-0.59</td>
<td>-1.67</td>
<td>-1.34</td>
<td>-1.36</td>
</tr>
<tr>
<td>Sulbactam (8 mg/liter)</td>
<td>-0.08</td>
<td>0.03</td>
<td>-0.12</td>
<td>-0.29</td>
<td>-1.39</td>
</tr>
<tr>
<td>Sulbactam (32 mg/liter)</td>
<td>-0.03</td>
<td>-0.07</td>
<td>-1.11</td>
<td>-1.96</td>
<td>-1.77</td>
</tr>
</tbody>
</table>

\text{a} Green shading indicates } >2 \log_{10} \text{ more killing than that achieved with the most active monotherapy (i.e., values meet the empirical definition of synergy), blue shading indicates } 1.0 \text{ to } 2.0 \log_{10} \text{ more killing than the most active monotherapy, and purple shading indicates } 0.5 \text{ to } 1.0 \log_{10} \text{ more killing than the most active monotherapy.}

Mechanistic synergy. For \textit{A. baumannii} strains ATCC 19606 and FADDI-AB034, subpopulation synergy alone was not sufficient to describe the extensive and synergistic killing by the combinations. Population predictions for the treatment arms containing 4 mg/liter aminoglycoside were considerably better for models with mechanistic synergy than for models without this feature. A thorough modeling analysis identified tobramycin enhancing the
target site penetration of imipenem as the most likely additional synergy mechanism. This was expressed as smaller KC_{50,IR,IPM} and KC_{50,R,IPM} in the presence of at least 4 mg/liter aminoglycoside (Fig. 1B). The imipenem concentration yielding half-maximal killing of the aminoglycoside-resistant population (KC_{50,R,IPM}) decreased approximately 4-fold in the presence compared to the absence of at least 4 mg/liter aminoglycoside (P < 0.0001 for FADDI-AB034 and P = 0.015 for ATCC 19606; likelihood ratio test) (Table 3). Additionally, the aminoglycoside (Fig. 1B) reduced the imipenem concentration, yielding half-maximal killing of the imipenem-resistant population (KC_{50,R,IPM}) from 16.5 to 13.2 mg/liter (P = 0.015) for strain ATCC 19606 (Table 3); this contributed considerably to killing of this population by imipenem. Models with subpopulation and mechanistic synergy yielded unbiased and precise curve fits for strains ATCC 19606 and FADDI-AB034 (Fig. 2 and 3).

Monte Carlo simulations. For 7 mg/kg tobramycin given as a 0.5-h infusion, the predicted median concentrations (5th to 95th percentiles) were 17.7 (12.8 to 23.8) mg/liter at 0.5 h, 15.0 (11.5 to 19.1) mg/liter at 1.0 h, and 1.43 (0.583 to 2.76) mg/liter at 24 h. A continuous infusion of 4 g/day imipenem yielded an unbound steady-state concentration of 13.4 (7.61 to 22.6) mg/liter (Fig. 4A). The imipenem concentration yielding half-maximal killing, with extensive regrowth of resistant bacteria (Fig. 4C and D). This highlights the need to rationally optimize combinations. Imipenem combinations provided the most extensive killing (>5 log_{10}) killing without regrowth for all simulated combination dosage regimens. However, combination regimens with intermittent dosing of 1 g imipenem every 6 or 8 h plus 5 or 7 mg/kg tobramycin were predicted to have 9.3% to 39.1% of patients failing, with extensive regrowth of resistant bacteria (Fig. 4C and D). In contrast, a continuous infusion of 4 g/day imipenem plus 7 mg/kg tobramycin was predicted to achieve >5 log_{10} without regrowth in 98.2% of the patients and was thus the most robust dosage regimen (Fig. 4D). The predicted success rate decreased from 98.2% to 92.8% if the tobramycin dose was reduced to 5 mg/kg per day and to 93.2% if the imipenem continuous infusion dose was reduced to 3 g per day (Fig. 4), since fewer patients achieved unbound-imipenem steady-state concentrations above 8 mg/liter.

**DISCUSSION**

The present study demonstrates that *A. baumannii* is extremely difficult to kill by monotherapy with any β-lactam or aminoglycoside. *In vitro* time-kill studies showed that at the highest clinically relevant unbound-steady-state concentration, all tested monotherapies achieved limited killing against strain ATCC 19606 at a high inoculum (Table 1). Imipenem was the most active monotherapy, whereas noncarbapenem β-lactams and aminoglycosides achieved much less killing, which was followed, for aminoglycosides, by extensive regrowth. This highlights the need to rationally optimize combinations. Imipenem combinations provided the most extensive killing and were more synergistic in comparison to all other tested β-lactam-plus-aminoglycoside combinations against ATCC 19606 (Table 1 and 2). Therefore, imipenem-plus-aminoglycoside combinations were progressed to further studies with three clinical isolates. Imipenem plus an aminoglycoside at clinically relevant

**TABLE 2** Log_{10} change in viable counts compared to 0-h counts for *A. baumannii* ATCC 19606 in static-concentration time-kill studies comparing four carbapenems with or without tobramycin at an initial inoculum of 10^{7.0} CFU/ml

<table>
<thead>
<tr>
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<th>1.5 h</th>
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<tbody>
<tr>
<td>Growth control</td>
<td>0.23</td>
<td>0.83</td>
<td>1.26</td>
<td>1.89</td>
<td>1.53</td>
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<tr>
<td>Tobramycin (4 mg/liter)</td>
<td>-1.89</td>
<td>-2.04</td>
<td>-2.10</td>
<td>1.21</td>
<td>1.20</td>
</tr>
<tr>
<td>Imipenem (8 mg/liter)</td>
<td>-2.61</td>
<td>-3.96</td>
<td>-4.58</td>
<td>-4.29</td>
<td>-4.73</td>
</tr>
<tr>
<td>Biapenem (8 mg/liter)</td>
<td>-2.15</td>
<td>-3.84</td>
<td>-4.32</td>
<td>-4.40</td>
<td>-4.50</td>
</tr>
<tr>
<td>Doripenem (8 mg/liter)</td>
<td>-1.93</td>
<td>-2.77</td>
<td>-3.95</td>
<td>-3.47</td>
<td>-2.95</td>
</tr>
<tr>
<td>Meropenem (8 mg/liter)</td>
<td>-1.36</td>
<td>-2.64</td>
<td>-2.99</td>
<td>-2.66</td>
<td>-3.70</td>
</tr>
<tr>
<td>Imipenem (8 mg/liter) + tobramycin (4 mg/liter)</td>
<td>-3.29</td>
<td>-4.28</td>
<td>≤-6.80</td>
<td>≤-6.80</td>
<td>-7.02</td>
</tr>
<tr>
<td>Biapenem (8 mg/liter) + tobramycin (4 mg/liter)</td>
<td>-3.10</td>
<td>-3.92</td>
<td>-5.32</td>
<td>-4.42</td>
<td>-5.57</td>
</tr>
<tr>
<td>Doripenem (8 mg/liter) + tobramycin (4 mg/liter)</td>
<td>-2.81</td>
<td>-3.78</td>
<td>-5.42</td>
<td>-5.01</td>
<td>-4.75</td>
</tr>
<tr>
<td>Meropenem (8 mg/liter) + tobramycin (4 mg/liter)</td>
<td>-2.68</td>
<td>-3.33</td>
<td>-4.19</td>
<td>-2.80</td>
<td>-4.75</td>
</tr>
</tbody>
</table>

* To achieve a limit of counting of 0.22 log_{10} CFU/ml (equivalent to one colony over 6 agar plates), in total 600 µl of this bacterial suspension was plated on six agar plates (100 µl per plate).
* A sterility check was performed by plating the entire volume of the bacterial suspension at the end of the experiment. The sterility check demonstrated that all bacteria were killed (i.e., zero colonies observed after plating the entire bacterial suspension).
* Green shading indicates >2 log_{10} more killing than that achieved with the most active monotherapy (i.e., values meet the empirical definition of synergy), blue shading indicates 1.0 to 2.0 log_{10} more killing than the most active monotherapy, and purple shading indicates 0.5 to 1.0 log_{10} more killing than the most active monotherapy.
### TABLE 3
Population parameter estimates for imipenem plus aminoglycoside combination models against four *A. baumannii* strains

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol (unit)</th>
<th>Mean value (SE%) for strain with drugs$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log$_{10}$ initial inoculum</td>
<td>$\text{CFU}_0$</td>
<td>ATCC 19606; IPM + AGS (TOB, ISE, and AMK) 7.99 (2.3); 6.87 (1.6)$^a$</td>
</tr>
<tr>
<td>Log$_{10}$ maximum population size</td>
<td>$\text{CFU}_{\text{max}}$</td>
<td>FADDI-AB016; IPM + AGS (ISE) 9.65 (2.3)</td>
</tr>
<tr>
<td>Replication rate constant</td>
<td>$k_1$ (h$^{-1}$)</td>
<td>50 (fixed)</td>
</tr>
<tr>
<td>Mean generation time</td>
<td>$k_{12,SS}$ (min)</td>
<td>96.4 (11.2)</td>
</tr>
<tr>
<td>Log$_{10}$ mutation frequency</td>
<td>$\text{MUT}_{\text{IPM}}$</td>
<td>−3.16 (5.8) 6.71 (3.8) 6.71 (3.8) 6.62 (2.8)</td>
</tr>
<tr>
<td>Killing by imipenem</td>
<td>$K_{\text{max,IM}}$ (h$^{-1}$)</td>
<td>2.48 (12.4)</td>
</tr>
<tr>
<td>Imipenem concn causing 50% of $K_{\text{max,IM}}$</td>
<td>$K_{C50,SS,IPM}$ (mg/liter)</td>
<td>Mono, 1.01 (17.7); combo (AGS = 4 mg/liter), 0.971 (18.2)</td>
</tr>
<tr>
<td>Killing by aminoglycosides</td>
<td>$K_{\text{max,AGS}}$ (h$^{-1}$)</td>
<td>176 (11.4); combo, 0.422 (16.3)</td>
</tr>
<tr>
<td>Aminoglycoside concn causing 50% of $K_{\text{max,AGS}}$</td>
<td>$K_{C50,SS,AGS}$ (mg/liter)</td>
<td>Mono, 22.1 (44.2); combo, 147 (8.9)</td>
</tr>
<tr>
<td>Maximum turnover time for hypothetical signal molecules ($= 1/k_{\text{out,sig}}$)</td>
<td>MTT (h)</td>
<td>0.942 (14.1) 0.753 (32.9) 0.794 (35.8) 0.072 (31)</td>
</tr>
<tr>
<td>Maximum inhibition by hypothetical signal molecules</td>
<td>$I_{\text{max,sig12}}$</td>
<td>0.992 (9.6) 0.984 (18.4) 0.986 (21.4) 0.980 (20)</td>
</tr>
<tr>
<td>Log$_{10}$ of hypothetical signal molecule concn at 50% of max effect</td>
<td>$\text{IC}_{50,\text{sig}}$</td>
<td>7.61 (1.5) 8.03 (2.6) 8.01 (2.9) 8.10 (2.2)</td>
</tr>
<tr>
<td>Hill coefficient</td>
<td>$\text{Hill}_{\text{IPM}}$</td>
<td>IPM 5.0 (fixed)$^c$</td>
</tr>
<tr>
<td></td>
<td>$\text{Hill}_{\text{AGS}}$</td>
<td>AGS 2.71 (12.6)</td>
</tr>
<tr>
<td>SD of residual error on log$_{10}$ scale</td>
<td>SD$_{\text{CFU}}$</td>
<td>0.401 (7.3) 0.481 (11.0) 0.287 (15.6) 0.383 (9.6)</td>
</tr>
</tbody>
</table>

---

$^a$ Initial inocula from two different experiments. All other model parameter estimates were assumed to be the same at both initial inocula.

$^b$ The final model for isolate FADDI-AB034 used the same parameter estimate for $K_{C50,SS,IPM}$ and $K_{C50,RI,IPM}$ with and without the aminoglycoside, since allowing for different parameter estimates yielded no improvement. The model benefitted significantly from using different estimates for $K_{C50,IR,IPM}$ for monotherapy and combination therapies.

$^c$ We used only three imipenem concentrations in each of the static-concentration time-kill studies. Therefore, precise estimation of the Hill coefficients was difficult. It was, however, beneficial to include a Hill coefficient for imipenem. After evaluation of different Hill coefficient values, the Hill coefficients were fixed at the values shown here.

$^d$ SE%, relative standard error, in percent; mono, monotherapy; combo, combination therapy.
concentrations achieved extensive killing (>5 log10) and completely prevented regrowth against a high inoculum of three carbapenem-intermediate or carbapenem-resistant A. baumannii isolates (Fig. 2). Overall, imipenem combinations eradicated three of four strains. We did not quantify resistant bacteria on antibiotic-containing agar plates and did not characterize the potential emergence of a new resistance mechanism(s) during antibiotic exposure. However, eradication suggested that imipenem-plus-aminoglycoside combinations killed resistant bacteria and prevented emergence of resistance.

Our results considerably extend those from the relatively few published studies on β-lactam-plus-aminoglycoside combinations in A. baumannii. Checkerboard studies at low inocula showed promising results for these combinations (16, 39–41), and SCKT experiments showed extensive killing (12, 15, 42–45) but also revealed regrowth of A. baumannii by several log10 for some combinations (12, 45). Cefepime plus amikacin was synergistic in SCKT experiments and yielded approximately 1 log10 more killing than cefepime monotherapy but was followed by approximately 6 log10 regrowth at 48 h in a hollow-fiber infection model (12). This combination was effective in a mouse pneumonia model (cefepime given every 8 h plus amikacin every 24 h; monotherapies not studied) (46). An empirical, nonoptimized combination regimen with imipenem plus tobramycin (both given every 6 h in mice) yielded 5.5 log10 killing of two carbapenem-resistant A. baumannii isolates in a mouse pneumonia model (47). All but one of the published SCKT studies (15) assessed monotherapies and combinations for only one β-lactam or one aminoglycoside, and no study on A. baumannii applied time course modeling (12, 15, 42–47). We identified synergistic combinations of 10 clinically important β-lactams and three aminoglycosides and used novel mechanism-based modeling and Monte Carlo simulations to rationally optimize combination dosage regimens for future studies in animals and humans.

To quantify the extent, time course, and potential mechanisms of synergy, we developed new mechanism-based models for four A. baumannii strains. The proposed model structure and parameter estimates were consistent across all strains (Fig. 1B; Table 3). Each model contained a susceptible population, an imipenem-resistant population which was slowly replicating (resembling persisters), and an aminoglycoside-resistant population. The aminoglycoside-resistant population (Fig. 1B) was killed by imipenem, whereas aminoglycoside-related killing of the imipenem-resistant population was relatively slow (as shown by K\text{max,RLAGS}) (Table 3) potentially due to a lower rate of protein synthesis. Future studies (for example, using a green-fluorescent-protein-labeled A. baumannii strain) would be required to experimentally prove the presence of persisters. Models with subpopulation synergy (i.e., imipenem killing the aminoglycoside-resistant population and vice versa) excellently described the in vitro viable-count profiles for strains FADDI-AB014 and FADDI-AB016 (Fig. 2 and 3).

To describe the extensive synergistic killing of strains ATCC 19606 and FADDI-AB034 by imipenem-plus-aminoglycoside combinations, an additional synergy mechanism was required. Modeling suggested that the aminoglycoside most likely enhanced the target site concentrations of imipenem. This mechanism is in agreement with studies using an albumin-conjugated aminoglycoside that disrupts the outer membrane of P. aeruginosa (33, 34). The outer membrane presents a major penetration barrier, particularly in A. baumannii (21, 22), and its disruption likely yields higher imipenem concentrations at the periplasmic target site and thus increased killing by imipenem.

The presence of 4 mg/liter aminoglycoside was modeled to significantly enhance the target site penetration of imipenem for the imipenem-resistant and the aminoglycoside-resistant populations (Fig. 1B; Table 3). This is a plausible mechanism, since it is the extracellular aminoglycoside concentration that likely causes disruption of the outer membrane. Mechanism-based models with subpopulation and mechanistic synergy excellently described...
Simulated plasma concentrations and viable-count profiles of the imipenem-resistant (MIC, 32 mg/liter) isolate A. baumannii FADDI-AB034 for monotherapy and combination dosage regimens. These Monte Carlo simulations predicted bacteremia in critically ill patients with normal renal function who completely lacked any effect of the immune system. The success rate ($P_{\text{success}}$, defined as $\leq 6 \log_{10} \text{CFU/ml}$ at 168 h) is indicated for each simulated dosage regimen. All imipenem infusions were given over 1 h (except for the continuous infusion). Tobramycin was infused over 0.5 h. The initial inoculum was $10^6.6$ CFU/ml.

FIG 4 Simulated plasma concentrations and viable-count profiles of the imipenem-resistant isolate A. baumannii FADDI-AB034 for monotherapy and combination dosage regimens. These Monte Carlo simulations predicted bacteremia in critically ill patients with normal renal function who completely lacked any effect of the immune system. The success rate ($P_{\text{success}}$, defined as $\leq 6 \log_{10} \text{CFU/ml}$ at 168 h) is indicated for each simulated dosage regimen. All imipenem infusions were given over 1 h (except for the continuous infusion). Tobramycin was infused over 0.5 h. The initial inoculum was $10^6.6$ CFU/ml.
all viable-count profiles for strains ATCC 19606 and FADDI-AB034 (Fig. 2 and 3).

These novel mechanism-based models were used together with population pharmacokinetic models for critically ill patients (17, 26, 27) to predict the time course of bacterial counts for infections in humans. Our simulations are based on in vitro time-kill studies with static antibiotic concentrations. In a previous study on ceftriaxone against *P. aeruginosa* (31), we developed a mechanism-based model using static concentration time-kill data and showed that this model successfully predicted the time course of viable counts in dynamic in vitro infection models from eight published studies (31). Additional Monte Carlo simulations (19) showed that this model successfully predicted the targets for cephalosporins for stasis to $1 \log_{10}$ killing (40% time of unbound drug concentrations above MIC [$fT_{\geq \text{MIC}}$]) and near-maximal bacterial killing at 24 h (60 to 70% $fT_{\geq \text{MIC}}$) in mice (48) and the targets for successful therapy in patients (49). Our in vitro experiments and mechanism-based models do not include the effect of the immune system (50, 51) and thus mirror immunocompromised patients. Acknowledging the uncertainty arising from these potential limitations, mechanism-based Monte Carlo simulations can predict and rationally optimize bacterial killing and prevention of resistance for combination dosage regimens (19, 31, 38, 52).

Our Monte Carlo simulations were performed using the carbapenem-resistant clinical isolate FADDI-AB034 at a high inoculum. This strain had an imipenem MIC of 32 mg/liter, which is the 97th percentile of the MIC distribution for *A. baumannii* by EUCAST and thus represents a near-worst-case scenario. The target population was comprised of critically ill patients with normal renal function; these patients were assumed to completely lack any effect of the immune system and to have bacteremia. As imipenem and tobramycin clearance had coefficients of variation of 34% and 31% and imipenem had a large variability (81%) for volume of distribution of the central compartment in critically ill patients (26, 27), Monte Carlo simulations predicted a large between-patient variability in the unbound plasma concentrations and viable counts (Fig. 4). While all monotherapies were predicted to fail in $>95\%$ of patients (Fig. 4B), the extensive synergy between imipenem and tobramycin was highly beneficial for all combination regimens. Combinations with 7 mg/kg tobramycin (Fig. 4D) displayed more rapid killing than combinations with 5 mg/kg tobramycin (Fig. 4C).

Combinations with short-term imipenem infusions of 1 g every 6 or 8 h were predicted to fail with extensive regrowth of resistant bacteria in 9.3% to 39.1% of patients. For continuous infusion of 3 g/day imipenem (with a 1-g loading dose) combined with 5 or 7 mg/kg tobramycin, 6.8% to 16.2% of patients were predicted to fail, with extensive regrowth (Fig. 4C and D). Excitingly, imipenem at 4 g/day as a continuous infusion (with a 1-g loading dose) plus tobramycin at 7 mg/kg every 24 h was predicted to yield extensive killing ($>5 \log_{10}$) without regrowth in 98.2% of patients (i.e., regrowth in only 1.8% of patients) (Fig. 4D). This highlights the importance of evaluating the robustness of combination dosage regimens via Monte Carlo simulations in the presence of the large between-patient variability in pharmacokinetics (17, 19, 53, 54).

While imipenem at 4 g/day clearly benefited the predicted success rate of therapy, this dose may slightly increase the risk of seizures compared to 2 or 3 g imipenem per day (55, 56). To obtain extensive synergy with tobramycin and prevent resistance, an unbound imipenem concentration of approximately 8 mg/liter was needed against our carbapenem-resistant isolates. Therapeutic drug monitoring may be valuable to achieve unbound steady-state concentrations of at least 8 mg/liter imipenem using 2 or 3 g imipenem per day.

In summary, bacterial killing by any β-lactam or aminoglycoside in monotherapy was limited against a high inoculum of wild-type *A. baumannii* ATCC 19606. Among all tested combinations, imipenem plus an aminoglycoside provided the most extensive killing without regrowth against high inocula of susceptible, carbapenem-intermediate, and carbapenem-resistant strains. Mechanism-based modeling identified both subpopulation synergy and mechanistic synergy for imipenem–plus-aminoglycoside combinations. This study presents the first application of Monte Carlo simulations for *A. baumannii* that were used to rationally optimize combination dosage regimens based on human population pharmacokinetics. Monte Carlo simulations predicted a 98.2% success rate for clinically relevant imipenem-plus-tobramycin combination dosage regimens against a carbapenem-resistant clinical *A. baumannii* isolate with an MIC of 32 mg/liter. This strongly suggests the future evaluation of these highly promising combination dosage regimens.

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We have no conflicts of interest to declare.

REFERENCES


