Quantitative Assessment of Combination Antimicrobial Therapy against Multidrug-Resistant Bacteria in a Murine Pneumonia Model

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Background. Combination antimicrobial therapy is clinically used as a last-resort strategy to control multidrug-resistant bacterial infections. However, selection of antibiotics is often empirical, and conventional assessment of combined drug effect has not been correlated to clinical outcomes. Here, we report a quantitative method to assess combined killing of antimicrobial agents against 2 multidrug-resistant bacteria.

Methods. Combined time-kill studies were performed using clinically achievable concentrations for each 2-agent combination against clinical isolates of Acinetobacter baumannii and Pseudomonas aeruginosa. Bacterial burden observed at 24 h was mathematically modeled using a 3-dimensional response surface. Subsequently, a neutropenic murine pneumonia model with simulated clinical dosing exposures was used to validate our quantitative assessment of combined killing.

Results. Different antimicrobial combinations were found to have varying efficacy against the multidrug-resistant bacteria. As predicted by our quantitative method, cefepime plus amikacin was found to be the most superior combination, which was evidenced by a reduction in tissue bacterial burden and prolonged survival of infected animals.

Conclusions. The consistency between the predictions of the mathematical model and in vivo observations substantiated the robustness of our quantitative method. These data highlighted a novel and promising method to guide rational selection of antimicrobial combination in the clinical setting.

Prevalence of nosocomial infections due to multidrug-resistant (MDR) gram-negative bacteria has increased over the past decade and is a major concern among hospitalized patients throughout the world. Acinetobacter baumannii and Pseudomonas aeruginosa are 2 particularly problematic MDR pathogens [1] and are known to be resistant to almost all commercially available antibiotics. Infections due to A. baumannii and P. aeruginosa are associated with severe illness [2, 3], and the mortality rate associated with these MDR bacteria among hospitalized patients has increased dramatically in recent years [4–6].

Treatment of infections due to MDR pathogens often represents a major challenge to clinicians, because most antibiotics are ineffective when used alone. Combination antimicrobial therapy is frequently considered as the last viable therapeutic strategy to control and suppress MDR pathogens. To date, the antibiotics to be chosen in combination antimicrobial therapy are often empirically selected by clinicians on the basis of intuition and anecdotal reports. This empirical approach is poorly guided, and the combination therapy selected may not always be optimal for patient care, because different combinations of antibiotics may be associated with different killing activity against MDR pathogens. If the combination of ≥2 antibiotics can achieve synergy, it is expected that this combination would have enhanced antibacterial activity. However, antagonism...
has been observed between different antibiotics, which can result in a decrease in overall antibacterial effect [7, 8]. More importantly, assessment of combined drug effect based on conventional methods (eg, checkerboard method) has not been correlated to clinical outcomes [9, 10]. A reliable method to quantify the combined effects of antibiotics will undoubtedly benefit clinicians in selecting combination therapy for MDR infections.

Previously we developed a mathematical model to examine the combined activity of antibiotics and provided useful background information to facilitate selection of combination therapy [11]. This model has been validated using an in vitro infection model against a clinical MDR A. baumannii strain [12]. However, in vivo relevance of this method has not been established. The objective of this study was to validate the mathematical model in a neutropenic murine pneumonia model in which combination therapy was used. The consistency between predictions of the mathematical model and in vivo observations will substantiate the robustness of our model. It is hoped that this model-based screening method would have the capability to examine a large number of antimicrobial agent combinations efficiently and to provide insights of their effectiveness. Consequently, it may guide clinicians in selecting the most effective combination(s) in the clinical setting.

MATERIALS AND METHODS

Bacteria. Two clinical MDR bacterial isolates were used in this study. The A. baumannii strain (AB1261 belonging to the ACB-20 clone) was obtained from a recent outbreak in Chicago, Illinois. Previous molecular investigations revealed that the isolate harbored OXA-40 (a carbapenem-hydrolyzing oxacillinase), a weak AmpC, and an unspecified TEM-ß-lactamase [13]. The minimum inhibitory concentrations (MICs) of cefepime, amikacin, and levofloxacin for this strain were 32 mg/L, 256 mg/L, and 32 mg/L, respectively. The bacteria were stored at −70°C in Protect (Key Scientific Products) storage vials. Fresh isolates were subcultured twice on 5% blood agar plates (Hardy Diagnostics) for 24 h at 35°C prior to each experiment.

Antimicrobial agents. For in vitro investigations, cefepime, amikacin, and levofloxacin were obtained from Bristol-Myers Squibb Research Institute, LKT Laboratories, and Johnson & Johnson Pharmaceutical Research & Development, respectively. A stock solution of each antimicrobial agent in sterile water was prepared, aliquoted, and stored at −70°C. For in vivo experiments, cefepime, amikacin, and levofloxacin were obtained from Apotex, Sicor Pharmaceuticals, and Ortho-McNeil Pharmaceuticals, respectively. Each antimicrobial agent was reconstituted in water for injection and diluted accordingly prior to each experiment.

Combined time-kill studies and modeling. Assessment of in vitro killing of different antimicrobial combinations against AB1261 has been reported elsewhere [12]. A similar approach was used for PA9019. Briefly, combined time-kill studies for PA9019 were performed using 25 concentration combinations in a 5 × 5 array for each 2-agent combination. The concentration ranges examined were constrained to those clinically achievable, and specific concentrations investigated were predetermined on the basis of similar prior single-agent time-kill studies. Total bacterial burden at 24 h (in triplicate) was determined by quantitative culture, and the data were mathematically modeled using a 3-dimensional response surface as described in detail elsewhere [11]:

\[
\log_{10}\left(\frac{[\text{CFU}]}{\text{mL}}\right) = Z_{\text{intercept}} - \left(\frac{E_{\text{maxA}} C_{\text{A}}^H + E_{\text{maxB}} C_{\text{B}}^H}{E_{\text{maxA}} C_{\text{A}}^H + E_{\text{maxB}} C_{\text{B}}^H}\right),
\]

where \(\log_{10}\left(\frac{[\text{CFU}]}{\text{mL}}\right)\) refers to the size of the bacterial population at 24 h and CFU stands for colony-forming units; \(Z_{\text{intercept}}\) is the bacterial density at 24 h in the absence of drug; \(E_{\text{maxA}}\) and \(E_{\text{maxB}}\) are the maximal effects of drugs A and B, respectively; \(C_{\text{A}}\) and \(C_{\text{B}}\) are the concentrations of drugs A and B, respectively; \(H_{\text{A}}\) and \(H_{\text{B}}\) are Hill-like exponents that determine how sigmoidal the effects of drugs A and B are, respectively; and \(C_{\text{A}}^{\text{max}}\) and \(C_{\text{B}}^{\text{max}}\) are the concentrations of drugs A and B required to achieve 50% of the maximal effect, respectively.

The predicted and observed volumes under the plane (VUP) were derived by double integration (with respect to concentration of both drugs) and interpolation of the experimental data, respectively. Confidence intervals (95% CIs) of the observed VUP (VUP_{observed}) were computed with mean data points (±2.56 standard deviations [SDs]). Synergy and antagonism were defined as interaction index (VUP_{observed}/VUP_{predicted}) of <1 and >1, respectively.

Animals. Female Swiss Webster mice weighing 20–25 g (Harlan Sprague-Dawley) were used for this study. The animals were housed in negative-pressure ventilated micro-isolator cages to decrease the risk of infection from extraneous path-
Assessment of Combination Therapy

Figure 1. Graphical representation of assessment of various antimicrobial agent combinations; cefepime plus amikacin (A), cefepime plus levofloxacin (B), levofloxacin plus amikacin (C). Time-kill studies were performed using 25 concentration combinations in a 5 × 5 array for each antimicrobial agent combination, constraining to clinically achievable unbound concentration ranges: cefepime (0–200 mg/L), amikacin (0–80 mg/L), and levofloxacin (0–9 mg/L). Specific concentrations used were predetermined to capture the most precise killing at 24 h. The red mesh surface is the expected killing of the antimicrobial agent combination (when there is a lack of significant pharmacodynamic interaction between the 2 agents), where the solid dots are observed killing. When a dot is below the red mesh surface, observed killing is greater than expected killing (ie, synergism). On the other hand, when a dot (shown in red with a green drop-down line) is above the red mesh surface, observed killing is less than expected killing (ie, antagonism). Furthermore, the vertical distance between the experimental datum point and the expected killing surface could be used as an index of interaction (the further the distance, the greater the synergy or antagonism). With multiple concentration combinations, overall killing over the concentration ranges examined is represented by the volume under the plane (VUP), which is a 3-dimensional analogy of the more widely recognized term area under the curve. The interactive index was derived by computing the ratio of the VUP between the observed and expected surfaces: \( \frac{\text{VUP}_{\text{observed}}}{\text{VUP}_{\text{expected}}} \). Synergy and antagonism were defined as interaction index <1 and >1, respectively. As shown above, an enhanced combined killing effect was seen with the cefepime-amikacin combination (A). In contrast, there was marginal decrease in the combined killing effect of the amikacin-levofloxacin combination (C). Assessment and ranking of combined killing of various antimicrobial agent combinations are as shown Table 1. CFU, colony-forming units.

ogens. The mice were allowed to eat and drink ad libitum. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Houston.

Experimental pneumonia model. To reduce the influence of the innate immune function on the observed outcomes, transient neutropenia was induced using 2 doses of intraperitoneal cyclophosphamide: 150 mg/kg administered 4 days prior to infection and 100 mg/kg administered 1 day prior to infection. This procedure resulted in transient neutropenia that persisted for 1 week after the last injection [14]. In addition, tran-
sient nephrotoxicity was induced by an intraperitoneal injection of uranyl nitrate at a dosage of 5 mg/kg 2 days prior to infection. This procedure resulted in transient renal failure that persisted for 5–6 days after the injection [15]. The animals were anesthetized by a single intraperitoneal injection of 1.25% 2,2,2-tribromoethanol at a dosage of 250 mg/kg (Sigma-Aldrich). An overnight culture was inoculated in cation-adjusted Mueller-Hinton broth (BBL), grown to log phase growth and diluted accordingly on the basis of absorbance at 630 nm. The bacteria were washed once in sterile saline and were inoculated (10 μL) into the trachea of anesthetized animals under laryngoscopic guidance.

**Lethal inoculum studies.** To adjust for different bacterial virulence, mice were infected as outlined above. For each bacterium, 39 mice were randomly divided into 3 groups, with inocula ranging from $1 \times 10^5$ CFU to $1 \times 10^8$ CFU. In each group, 3 mice were sacrificed at baseline to ascertain the infective inoculum, and 10 mice were observed every 8 h for up to 4 days. The optimal inoculum that resulted in 50%–100% mortality between 32 and 72 h was used in the following studies. From the clinical perspective, these experimental conditions mimic a window of opportunity in which pharmacologic intervention might have an impact on patient outcomes.

**Immune response studies.** Twelve mice were randomly divided into 2 groups. Mice in the first group were infected with AB1261 as outlined above. Animals in the other (control) group were inoculated with sterile saline. All of the mice were sacrificed by CO₂ asphyxiation 24 h after infection, and blood was collected via cardiac puncture. The blood was allowed to clot on ice, and the serum was isolated by centrifugation at 4°C, 4000 g for 15 min. Serum tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) were assayed by enzyme-linked immunosorbent assay kits (BD Biosciences) according to the manufacturer’s instructions and were compared between the 2 groups.

**Pharmacokinetics.** Mice were infected as outlined above; 108 mice were randomly divided into 6 groups. Two hours after infection, 18 mice in each group were injected with a single dose (0.2 mL) intraperitoneally of each antimicrobial agent (cefepime, amikacin, or levofloxacin). The dosages administered were as follows: 120 and 240 mg/kg of cefepime, 7.5 and 30 mg/kg of amikacin, and 103 and 209 mg/kg of levofloxacin. At 0.5, 1, 2, 3, 4, and 6 h after injection, 3 mice per group were sacrificed, and blood was collected via cardiac puncture. The blood was allowed to clot on ice, and the serum was isolated by centrifugation. After protein precipitation with 5% methanol, drug concentrations in each sample were measured by a validated high-performance liquid chromatography or liquid chromatography-mass spectrometry methodology as detailed elsewhere [12]. The serum concentration profiles of cefepime, levofloxacin, and amikacin in infected mice were analyzed by fitting a 1-compartment model with first order absorption to the data. The best-fit model parameter estimates were subsequently used to determine the most appropriate dosages necessary to achieve a clinically relevant dose exposure (similar to humans). Drug binding to mouse serum proteins was not specifically investigated but was assumed to be similar to that for human serum proteins [16–20].

**Bacterial burden in lung tissue.** Bacterial burden in the animal lungs was determined 24 h after the start of antimicrobial therapy. Two hours after bacterial infection, 3 mice in the each treatment group were injected intraperitoneally (0.2 mL) with either (1) cefepime (180 mg/kg every 8 h) plus amikacin (20 mg/kg every 24 h); (2) cefepime plus levofloxacin (150 mg/kg every 24 h); or (3) amikacin plus levofloxacin.

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**Table 1. Assessment and Ranking of Combined Killing of Various Antimicrobial Agent Combinations against AB1261 and PA9019**

<table>
<thead>
<tr>
<th>Pathogen, antimicrobial combination</th>
<th>Interactive index (95% CI)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AB1261</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefepime plus amikacin</td>
<td>0.698 (0.675–0.722)</td>
<td>Synergism</td>
</tr>
<tr>
<td>Cefepime plus levofloxacin</td>
<td>0.929 (0.903–0.956)</td>
<td>Synergism</td>
</tr>
<tr>
<td>Amikacin plus levofloxacin</td>
<td>0.994 (0.982–1.005)</td>
<td>Additivity</td>
</tr>
<tr>
<td><strong>PA9019</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefepime plus amikacin</td>
<td>0.718 (0.677–0.758)</td>
<td>Synergism</td>
</tr>
<tr>
<td>Cefepime plus levofloxacin</td>
<td>1.020 (0.981–1.059)</td>
<td>Additivity</td>
</tr>
<tr>
<td>Amikacin plus levofloxacin</td>
<td>1.054 (1.094–1.014)</td>
<td>Antagonism</td>
</tr>
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**NOTE.** Synergy and antagonism were defined as interaction index $<1$ and $>1$, respectively. CI, confidence interval.  
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**Figure 2.** Survival of animals infected with AB1261 (A) and PA9019 (B) without treatment. CFU, colony-forming units.
Assessment of Combination Therapy

• JID 2010:201 (15 March) • 893

Figure 3. Comparison of serum tumor necrosis factor α (TNF-α) (A) and interleukin 6 (IL-6) (B) concentrations 24 h after infection.

Figure 4. Comparison of tissue bacterial burden before treatment was given (baseline), at the end of experiment (alive), and at the time of death (dead) for AB1261 (A) and PA9019 (B). Each datum point represents 1 infected animal; the horizontal line in each group represents the median bacterial burden of the group. For both bacteria, analysis of variance and Dunn’s multiple comparison test revealed that differences in bacterial burden between the alive and dead groups were statistically significant (P < .001). CFU, colony-forming units.

doses selected were guided by previous single-dose pharmacokinetic studies and were intended to produce unbound pharmacokinetic exposures similar to those associated with the usual maximum clinical dose. The mice were examined every 8 h. All infected mice were euthanized after 24 h by CO₂ asphyxiation, and lungs from each mouse were aseptically collected for quantitative culture. Prior to being cultured, lungs were homogenized in 10 mL of sterile saline. Lung homogenate suspensions were centrifuged (10°C at 4000 g for 15 min), decanted, and reconstituted with sterile saline at 10 times the original volume. The samples were subsequently serially diluted (×10) and plated on Mueller-Hinton Agar plates (Hardy Diagnostics). Colony counts were enumerated after incubation at 35°C in a humidified incubator for 24 h. The reliable lower limit of detection was 1000 CFU/g.

Survival. Two hours after infection, 52 mice received 2 of the 3 antimicrobial agents (combination therapy) at the doses described above. Three mice in each experiment were sacrificed at baseline to ascertain the infective inoculum. The mice were examined every 8 h. Moribund mice were humanely sacrificed at each inspection time, and death was recorded as occurred at the next inspection time. All mice were euthanized after 96 h by CO₂ asphyxiation. Lungs from each mouse were aseptically collected for quantitative culture as described previously, either upon death or at the end of the experiment.

Statistical analysis. Serum cytokines levels were analyzed using the Student t test. Bacterial burden in lung tissue was analyzed using 1-way analysis of variance (Kruskal-Wallis test). Survival was evaluated with the Kaplan-Meier survival analysis and log-rank test. A P value of <.05 was considered to be statistically significant.

RESULTS

Combined time-kill studies. In combination time-kill studies, an enhanced overall combined killing effect was seen when cefepime was used together with amikacin against both MDR bacteria (data with PA9019 are shown in Figure 1A). There was no considerable increase in the overall combined killing effect of the cefepime-levofloxacin combination for PA9019 (Figure 1B) and the amikacin-levofloxacin combination for AB1261.
Figure 5. Single-dose pharmacokinetics of the antimicrobial agents cefepime (A), amikacin (B), and levofloxacin (C) in infected animals. Data are presented as mean values (± standard deviations). Open symbols represent experimental observations. Dotted lines depict the best-fit models.

In contrast, there was a marginal decrease in the combined killing effect of the amikacin-levofloxacin combination against PA9019 (Figure 1C). Quantitative assessment of killing of various combinations is as shown in Table 1.

Validation of experimental pneumonia. In mice infected with $1 \times 10^7$ CFU of AB1261 and $5 \times 10^7$ CFU of PA9019, 50% of the mice expired 48 and 40 h after inoculation, respectively (Figure 2). Therefore, these lethal inocula were used in subsequent experiments. Serum TNF-α and IL-6 levels 24 h after infection were found to be significantly higher in infected mice than in normal control mice; mean TNF-α concentrations (± SD) were 45.4 ± 21.0 pg/mL vs 14.7 ± 4.3 pg/mL ($P = .015$) and IL-6 concentrations were 998.4 ± 122.4 pg/mL vs 87.2 ± 66.5 pg/mL ($P < .001$), respectively (Figure 3). These changes are consistent with the pathophysiology of acute pneumonia in humans [21].

For both MDR bacteria, an increase of tissue bacterial burden was found in dead animals, compared with baseline, with a count of $9.05 \pm 1.05$ vs $7.71 \pm 0.24 \log_{10}$ CFU/g for AB1261 ($P < .001$) and $9.05 \pm 1.11$ vs $3.37 \pm 0.24 \log_{10}$ CFU/g ($P < .001$) for PA9019. These results suggested that pneumonia was the primary cause of death in these animals. In addition, there was a significant difference in tissue bacterial burden between the live and dead animals (Figure 4).

Pharmacokinetics. The observed and best-fit pharmacokinetic profiles of various antimicrobial agents in infected animals are shown in Figure 5. All agents were rapidly absorbed, with the maximum serum concentrations achieved within 60 min. The pharmacokinetic model fits to the observed data were reasonable ($r^2 \geq 0.95$). Based on the best-fit model parameter estimates (Table 2), cefepime 180 mg/kg administered intraperitoneally 3 times daily, amikacin 20 mg/kg administered once daily, and levofloxacin 150 mg/kg administered once daily were used in subsequent experiments. These dosing regimens were selected to closely mimic drug exposures resulting from the intravenous clinical doses of cefepime (2 g every 8 h), amikacin (1500 mg every 24 h) and levofloxacin (750 mg every 24 h), respectively.

Bacterial clearance from lung. The tissue bacterial burdens observed at 24 h for each combination therapy are shown in Figure 6. The reduction in tissue bacterial burden was different with various agent combinations. Cefepime plus amikacin showed a greater lung clearance of AB1261 and PA9019 than the other 2 combinations. In contrast, there was no significant difference in lung clearance of PA9019 between the amikacin plus levofloxacin combination and the control group ($P > .05$). These observations were in general agreement with the mathematical model predictions (Table 1).

Survival. Survival of infected animals after treatment is shown in Figure 7. Similar to the tissue bacterial burden re-

### Table 2. Best-Fit Pharmacokinetic Model Parameter Estimates

This table is available in its entirety in the online version of the *Journal of Infectious Diseases*. 
Assessment of Combination Therapy

Infections caused by MDR gram-negative bacteria present daily challenges to clinicians worldwide. Unfortunately, the growing problem of multidrug resistance was not paralleled by the development of novel antimicrobial agents [22, 23]. As a result, there are now a growing number of reports of infection caused by MDR bacteria for which no adequate therapeutic option exists [24, 25]. The return to the preantibiotic era has become a reality in many parts of the world [26].

There is an urgent need for a concerted effort to develop new and effective treatment strategies. Combination therapy is

DISCUSSION

Infections caused by MDR gram-negative bacteria present daily challenges to clinicians worldwide. Unfortunately, the growing problem of multidrug resistance was not paralleled by the development of novel antimicrobial agents [22, 23]. As a result, there are now a growing number of reports of infection caused by MDR bacteria for which no adequate therapeutic option exists [24, 25]. The return to the preantibiotic era has become a reality in many parts of the world [26].

There is an urgent need for a concerted effort to develop new and effective treatment strategies. Combination therapy is

Figure 6. Comparison of tissue bacterial burden after 24 h of therapy for AB1261 (A) and PA9019 (B). Data are presented as mean values (± standard deviations). CFU, colony-forming units.

Figure 7. Survival of animals infected with AB1261 (A) and PA9019 (B) after treatment.

duction, different agent combinations were not equally efficacious. Against AB1261, cefepime plus amikacin demonstrated the most favorable survival rate over time, compared with the other combinations and the control group (P < .001). Against PA9019, the cefepime plus amikacin combination was also found to have the most favorable survival rate over time. Against both MDR bacteria, the amikacin plus levofloxacin combination was the least efficacious in prolonging survival. These observations were consistent with data on tissue bacterial burden reduction (in the previous section) and were again in general agreement with the mathematical model predictions (Table 1), validating its predicting ability in an in vivo system.
considered to be the only remaining viable therapeutic option [27–29]. Because the observed MDR phenotype may be mediated by several molecular resistance mechanisms, an antibiotic combination that has previously been found to be effective (ie, against the same pathogen isolated in another hospital) may not always be beneficial to another patient. In addition, monotherapy may not always be inferior to combination therapy [30, 31]. Certain antimicrobial agents may negate one another when combined, resulting in a reduced overall effect (ie, antagonism) [8, 32]. In this study, we briefly explored the utility of monotherapy against AB1261 (Figure 8). The results revealed that cefepime monotherapy (despite being considered resistant) demonstrated greater bacterial clearance in the lungs of infected animals, compared with the combination of amikacin plus levofloxacin ($P = .015$). Furthermore, the amikacin plus levofloxacin combination was not more efficacious in prolonging survival than was cefepime monotherapy ($P = .289$). It is clear that a reliable method to quantify the combined effects of multiple antimicrobial agents is necessary in selecting patient-specific combination therapy for MDR infections. However, a satisfactory methodology to objectively evaluate combination therapy and to quantify the extent of synergy and/or antagonism is currently not available. Consequently, it is difficult to compare different antimicrobial agent combinations in a rational manner.

Numerous studies have investigated the interaction between antimicrobial agents and combined antimicrobial effects. The checkerboard method and fractional inhibitory concentration index are the most widely used methodologies in studying the in vitro interaction of antimicrobial agents. This traditional approach relies on the assumption that inhibition of growth by antimicrobial agents follows a linear dose-response curve. It has been pointed out previously that the underlying assumption of the linear concentration-effect relationship may not be valid [33]; thus, the relevance of the results may be questionable and not predictive of clinical outcomes [9, 10]. An improved method needs to be developed [34]. Several interesting modeling approaches for assessing drug interactions have been put forth [35–37]. Although these methods are more complex and devoid of obvious limitations, conclusions regarding drug interactions have often not been prospectively validated. Therefore, the predictive ability of these methods has not been well established.

Circumventing the limitations of traditional methods, we developed a relatively simple approach to quantitatively assess the activity of 2 antimicrobial agents when used together. The experimental data are relatively simple to obtain, easily interpreted, and may be more applicable to a wide variety of clinical and experimental circumstances. Different pharmacological (eg, bactericidal or bacteriostatic activity) and microbiological characteristics of various drug-drug pathogen combinations could be adequately captured in their respective time-kill studies (without the need to have information on clonality and/or the specific mechanism[s] of multidrug resistance) and reflected in different estimates of the interactive index. More importantly, the results could be made available to clinicians in a timely manner (within days) for individualized bedside decisions. In this study, we used a neutropenic murine pneumonia model with simulated clinical dosing exposures to validate our quantitative assessment of combined killing. As predicted by our quantitative method, cefepime plus amikacin was found to be the most superior combination, which was evidenced by tissue bacterial burden reduction and prolonged survival of infected animals. The consistency between the predictions of the mathematical model and in vivo observations substantiated the robustness of our quantitative method. To the best of our knowledge, this is the first study in which a mathematical model could be limited in vitro data as inputs to make useful predictions regarding the in vivo effectiveness of various antimicrobial agent combinations against different MDR bacterial isolates.

The combined effect of antimicrobial agents is a complex biological system to study. Despite the fact that our model has been validated by using an in vitro and in vivo infection model, there are still several limitations to our study. In this study, we validated the mathematical model using only 2 representative MDR bacterial strains, a limited number of drug combinations, and only maximal doses approved by the US Food and Drug Administration. Although we have demonstrated the feasibility and utility of the prototype, the generalizability of the model would be more assured with clinical validation using more antimicrobial agents against a greater number of MDR bacterial strains. Also, the mathematical model used a single time point determination, and the dynamics of the bacterial population over time were not captured. Current work is in progress to address the change of population composition under various drug exposures. Finally, most of the experimental procedures were isolated. It is our vision that the practicality of introducing this new method into clinical practice would be optimally supported with an automated robotic testing system and integrated mathematical analysis.

In conclusion, we described a novel quantitative method to assess combined killing of antimicrobial agents against 2 MDR bacteria. Our approach appears to be robust, and further validation with other drug-drug-pathogen combinations is warranted.