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Paradoxical Effect of Polymyxin B: High Drug Exposure Amplifies Resistance in *Acinetobacter baumannii*

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Administering polymyxin antibiotics in a traditional fashion may be ineffective against Gram-negative ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) pathogens. Here, we explored increasing the dose intensity of polymyxin B against two strains of *Acinetobacter baumannii* in the hollow-fiber infection model. The following dosage regimens were simulated for polymyxin B ($t_{1/2}$ = 8 h): non-loading dose (1.43 mg/kg of body weight every 12 h [q12h]), loading dose (2.22 mg/kg q12h for 1 dose and then 1.43 mg/kg q12h), front-loading dose (3.33 mg/kg q12h for 1 dose followed by 1.43 mg/kg q12h), burst (5.53 mg/kg for 1 dose), and supraburst (18.4 mg/kg for 1 dose). Against both *A. baumannii* isolates, a rapid initial decline in the total population was observed within the first 6 h of polymyxin exposure, whereby greater polymyxin B exposure resulted in greater maximal killing of −1.25, −1.43, −2.84, −2.84, and −3.40 $\log_{10}$ CFU/ml within the first 6 h. Unexpectedly, we observed a paradoxical effect whereby higher polymyxin B exposures dramatically increased resistant subpopulations that grew on agar containing up to 10 mg/liter of polymyxin B over 336 h. High drug exposure also proliferated polymyxin-dependent growth. A cost-benefit pharmacokinetic/pharmacodynamic relationship between 24-h killing and 336-h resistance was explored. The intersecting point, where the benefit of bacterial killing was equal to the cost of resistance, was an $fAUC_{0-24}$ (area under the concentration-time curve from 0 to 24 h for the free, unbound fraction of drug) of 38.5 mg · h/liter for polymyxin B. Increasing the dose intensity of polymyxin B resulted in amplification of resistance, highlighting the need to utilize polymyxins as part of a combination against high-bacterial-density *A. baumannii* infections.

The polymyxin antibiotics have emerged as a last line of defense for the treatment of Gram-negative strains resistant to all other currently available antibiotics (1–3). Polymyxin B and colistin are often the only available agents with activity against these Gram-negative superbugs (4, 5). Even more worrisome is the first report of plasmid-mediated polymyxin resistance in *Escherichia coli*, which could be transferred to other Gram-negative strains (6). Furthermore, current dosage regimens for both polymyxin B and colistin result in polymyxin plasma concentrations that are suboptimal in a significant proportion of patients (7–10). *In vitro* and animal studies clearly demonstrate that antibiotic resistance is amplified during exposure to suboptimal polymyxin concentrations, especially against polymyxin-heteroresistant strains that are frequently isolated in *Acinetobacter baumannii* (11–14).

Administering nontraditional, high-intensity polymyxin regimens may be a strategy to maximize bacterial killing while minimizing resistance and potential toxicity. We have previously determined that front-loading colistin resulted in greater bacterial killing within the first 24 h against *Pseudomonas aeruginosa* (15). The polymyxins are suitable candidates for high-intensity exposure at the beginning of therapy due to their rapid bactericidal activity against high bacterial densities (16). However, high-intensity regimens for polymyxin B have not been evaluated against *A. baumannii*. To date, there have been no studies that profiled the time course of *A. baumannii* responses to polymyxin B over long-term durations at high bacterial densities that correspond to infections such as ventilator-associated pneumonia (VAP) (17, 18).

In the present study, we explored how increasing the dose intensity of polymyxin B influences bactericidal activity and resistance suppression against *A. baumannii*. A hollow-fiber infection model (HFIM) was used to simulate a range for polymyxin B varying in initial dose intensity. Bacterial killing and detailed population analysis profiles that tracked the amplification of resistance over a 14-day period were evaluated.

MATERIALS AND METHODS

Bacterial isolates, antibiotics, and susceptibility testing. Two *A. baumannii* strains were utilized, ATCC 19606 and 03-149.01. ATCC 19606 was selected as a laboratory control isolate. 03-149.01 was from a patient enrolled in an open-label population pharmacokinetic study who received colistin methanesulfonate as part of his or her clinical care for treatment of a bloodstream infection or pneumonia due to a Gram-negative bacillus (9). Neither isolate was previously exposed to polymyxin

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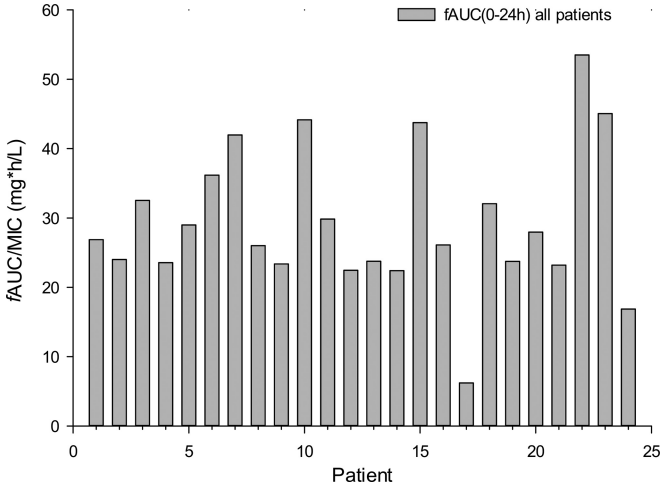

FIG 1 *f*AUCs over 0 to 24 h for polymyxin B for each of the 24 critically ill adult patients are shown and are based on the population pharmacokinetic model by Sandri et al. (7, 8). All patients received physician-selected, intravenous polymyxin B regimens ranging from 0.45 to 3.38 mg/kg/day. The resulting *f*AUCs are shown for each patient. In the previous population pharmacokinetic study, there were 23 patients who received the drug every 12 h and 1 patient who received polymyxin B every 24 h. The *f*AUC (0 to 24 h) ranged from 6.2 to 53.5 mg · h/liter with a median of 26.5 mg · h/liter, average of 29.4 mg · h/liter, and standard deviation of 10.4 mg · h/liter. The maximum *f*AUC over 24 h at steady state was 60.4 mg · h/liter. These data served as the basis for the simulated regimens to be studied in the hollow-fiber infection model.

B or colistin. Analytical-grade polymyxin B was purchased from Sigma-Aldrich (lot WXBB4470V; St. Louis, MO). Fresh stock solutions of polymyxin B were prepared immediately prior to each experiment. Cation-adjusted Mueller-Hinton broth (CAMHB) (Difco, Detroit, MI, USA) supplemented with calcium (25 mg/liter) and magnesium (12.5 mg/liter) was used as the growth medium. MIC values were determined in quadruplicate according to the CLSI guidelines. Both strains had a polymyxin B MIC of 0.5 mg/liter.

HFIM. The HFIM was used to simulate the time course of unbound plasma concentrations observed in patients receiving polymyxin B regimens by using an approach that was previously described (19, 20). Cellulosic hollow-fiber cartridges (C3008; Fiber Cell Systems, Fredrick, MD) were utilized in all experiments, and samples were serially collected for 336 h. Total population counts were quantified by depositing appropriately diluted bacterial samples on Mueller-Hinton agar (MHA) plates. In addition, population analysis profiles (PAP) were performed by plating diluted samples onto MHA plates containing polymyxin B at 0.5, 1, 2, 3, 4, 6, 8, or 10 mg/liter to track resistant subpopulations over 336 h.

Dosage regimens were simulated for polymyxin B ($t_{1/2}$ = 8 h) using a protein binding level of 42%, based on the population pharmacokinetic model by Sandri et al. (7, 8), from 24 adult patients that received physician-selected, intravenous polymyxin B dosage regimens ranging from 0.45 to 3.38 mg/kg/day. Of the 24 patients participating in the population pharmacokinetic study, 23 patients received the drug every 12 h and 1 patient received polymyxin B every 24 h. The following resultant $f\text{AUC}_{0-24}$ (area under the concentration-time curve from 0 to 24 h for the free, unbound fraction of drug) values were determined: range of 6.2 to 53.5 mg · h/liter, median of 26.5 mg · h/liter, average of 29.4 mg · h/liter, and standard deviations of 10.4 mg · h/liter, as shown in Fig. 1 (7). The maximum *f*AUC over 24 h at steady state was 60.4 mg · h/liter. There were 5 regimens simulated in the HFIM: regimen 1, polymyxin B non-loading dose (1.43 mg/kg every 12 h [q12h], generating an $f\text{AUC}_{0-24}$ of 28.9 mg · h/liter across the first day and an *f*AUC at steady state [$f\text{AUC}_{ss}$] of 35.9 mg · h/liter); regimen 2, polymyxin B with loading dose (2.22 mg/kg for 1 dose followed by 1.43 mg/kg q12h starting 12 h later, $f\text{AUC}_{0-24}$ of 35.9 mg · h/liter across each day); regimen 3, polymyxin B front-loading dose (3.33 mg/kg for 1 dose followed by 1.43 mg/kg q12h starting 12 h later, with an $f\text{AUC}_{0-24}$ of 48.2 mg · h/liter across the first day and an $f\text{AUC}_{ss}$ of 35.9 mg · h/liter at steady state); regimen 4, polymyxin B burst (5.53 mg/kg for 1 dose followed by no subsequent doses, with an $f\text{AUC}_{0-24}$ of 60.4 mg · h/liter); and regimen 5, polymyxin B supraburst (18.4 mg/kg for 1 dose followed by no subsequent doses, with an $f\text{AUC}_{0-24\text{ h}}$ of 202.5 mg · h/liter).

Four of the above-described regimens (non-loading dose [regimen 1], loading dose [2], front loading [3], and burst [4]) were in the range of the achieved *f*AUCs in critically ill patients from the population pharmacokinetic study of polymyxin B (7). Regimens 1 to 3 were administered across the 14-day HFIM study, while regimens 4 and 5 were administered as single-dose regimens given only on day 1. At steady state, the highest *f*AUC over 24 h achieved in critically ill patients was 60.4 mg · h/liter, which provided the rationale for simulating the polymyxin B burst regimen (regimen 4) that was administered as a single dose. Regimen 5, supraburst, was selected as a supratherapeutic proof-of-concept regimen to test the hypothesis that high-intensity regimens resulted in the amplification of resistance. The rationale for both of these regimens was to leverage the rapid, concentration-dependent, bactericidal activity of the polymyxin antibiotics at the beginning of therapy while decreasing potential exposure. Characterizing the pharmacodynamics of these new polymyxin regimens will be beneficial in defining how to optimally administer polymyxin B in combination with other antibiotics in the future. Polymyxin B concentrations were quantified using validated high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) as previously described (21), with good reproducibility (coefficients of variation of ≤9.0%) and accuracy (observed concentrations were ≤10% from target concentrations). The limit of quantification was 0.1 mg/liter.

Cost-benefit PK/PD analyses. To determine the pharmacokinetic/pharmacodynamic (PK/PD) relationship between exposure and bacterial killing and resistance, we utilized a cost-benefit approach. We defined the benefit as initial bacterial killing of the total population within the first 24 h and cost as the amplification of resistant subpopulations which included real-time PAP data over 336 h. An area-based integrated PK/PD measure was utilized as previously described (22). Using the linear trapezoid rule, the area under the CFU curve for the total population over 24 h (AUCFU_{0-24}) was calculated to assess bacterial killing. The 24-h log ratio area for bacterial killing then was calculated as the logarithm of the AUCFU_{0-24} of the total population ($\text{AUCFU}_{\text{drug total population}}$) divided by the AUCFU_{0-24} of the total population for the growth control ($\text{AUCFU}_{\text{control}}$), as described in equation 1. The AUCFU of resistant subpopulations was also calculated over 336 h ($\text{AUCFU}_{\text{resistant subpopulation}}$) and normalized by the AUCFU over 336 h of the total population ($\text{AUCFU}_{\text{total population}}$) to generate the 336-h log ratio area, a measure of resistance amplification (equation 2). For each polymyxin B regimen, five resistant subpopulations were used in the resistance analysis that were obtained from bacterial counts on agar containing 3, 4, 6, 8, and 10 mg/liter of polymyxin B (the CLSI breakpoint for polymyxin resistance is >2 mg/liter).

$$\text{24-h log ratio area}_{\text{bacterial killing}} = \log_{10}\left(\frac{\text{AUCFU}_{\text{drug total population}}}{\text{AUCFU}_{\text{control}}}\right) \quad (1)$$

$$\text{336-h log ratio area}_{\text{resistant subpopulation}} = \log_{10}\left(\frac{\text{AUCFU}_{\text{resistant subpopulation}}}{\text{AUCFU}_{\text{total population}}}\right) \quad (2)$$

$$E = E_0 - \frac{E_{\max} \times (f\text{AUC}_{24})^H}{(\text{EC}_{50})^H + (f\text{AUC}_{24})^H} \quad (3)$$

A four-parameter Hill-type model (equation 3) was fit to the effect parameter using Systat (version 12; Systat Software Inc., San Jose, CA) (22). In this model, E (dependent variable) represents the log ratio area, E_0 is the effect at a polymyxin B $f\text{AUC}_{24}$ of 0, $E_{\max}$ is the maximum effect of

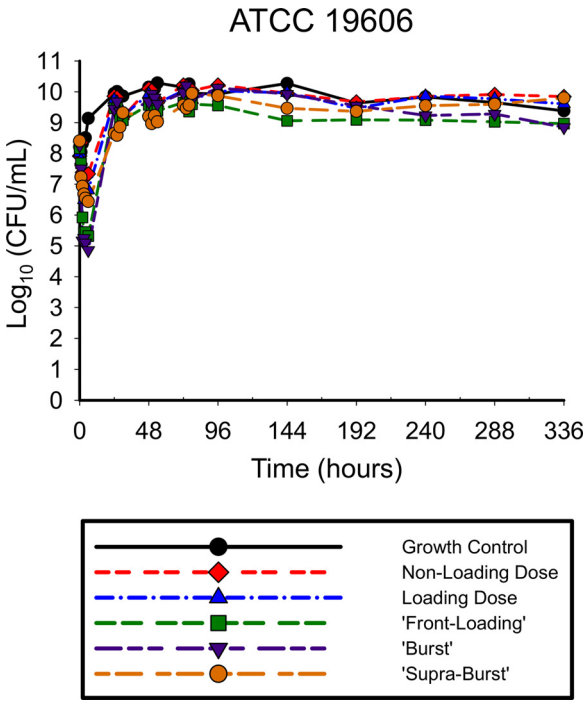

FIG 2 Total population of ATCC 19606 in response to polymyxin B regimens in the hollow-fiber infection model. The following regimens for polymyxin B were simulated: regimen 1, polymyxin B non-loading dose (1.43 mg/kg q12h, generating an $fAUC_{0-24}$ of 28.9 mg/liter · h across the first day and an $fAUC_{ss}$ of 35.9 mg · h/liter at steady state); regimen 2, polymyxin B with loading dose (2.22 mg/kg for 1 dose followed by 1.43 mg/kg q12h starting 12 h later, with an $fAUC_{0-24}$ of 35.9 mg · h/liter across each day); regimen 3, polymyxin B front-loading dose (3.33 mg/kg for 1 dose followed by 1.43 mg/kg q12h starting 12 h later, with an $fAUC_{0-24}$ of 48.2 mg · h/liter across the first day and an $fAUC_{ss}$ of 35.9 mg · h/liter at steady state); regimen 4, polymyxin B burst (5.53 mg/kg for 1 dose followed by no subsequent doses, with an $fAUC_{0-24}$ of 60.4 mg · h/liter); and regimen 5, polymyxin B supraburst (18.4 mg/kg for 1 dose followed by no subsequent doses, $fAUC_{0-24}$ of 202.5 mg · h/liter).

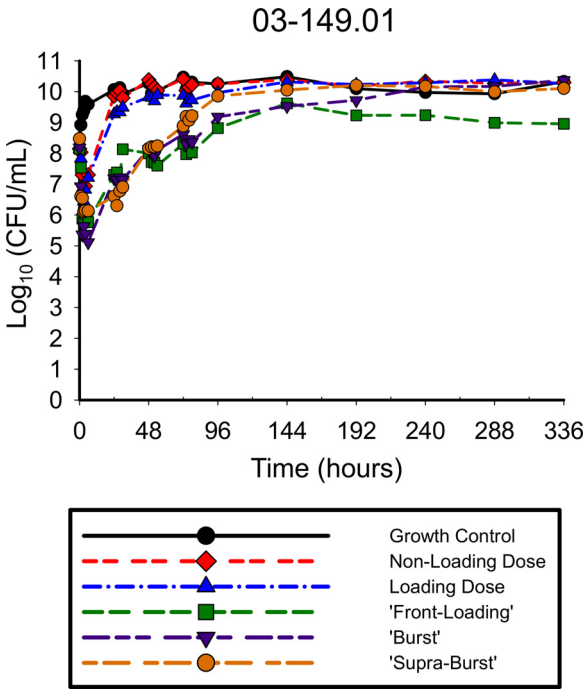

FIG 3 Total population of 03-149.01 in response to polymyxin B regimens in the hollow-fiber infection model. The following regimens for polymyxin B were simulated: regimen 1, polymyxin B non-loading dose (1.43 mg/kg q12h, generating an $fAUC_{0-24}$ of 28.9 mg/liter · h across the first day and an $fAUC_{ss}$ of 35.9 mg · h/liter at steady state); regimen 2, polymyxin B with loading dose (2.22 mg/kg for 1 dose followed by 1.43 mg/kg q12h starting 12 h later, with an $fAUC_{0-24}$ of 35.9 mg · h/liter across each day); regimen 3, polymyxin B front-loading dose (3.33 mg/kg for 1 dose followed by 1.43 mg/kg q12h starting 12 h later, $fAUC_{0-24}$ of 48.2 mg · h/liter across the first day and an $fAUC_{ss}$ of 35.9 mg · h/liter at steady state); regimen 4, polymyxin B burst (5.53 mg/kg for 1 dose followed by no subsequent doses, $fAUC_{0-24}$ of 60.4 mg · h/liter); and regimen 5, polymyxin B supraburst (18.4 mg/kg for 1 dose followed by no subsequent doses, $fAUC_{0-24}$ of 202.5 mg · h/liter).

the polymyxin B regimen, $fAUC_{24}$ is the free polymyxin B area under the curve within the first 24 h, EC_{50} is the polymyxin B $fAUC_{24}$ displaying 50% of the maximum effect, and H is the sigmoidicity constant.

RESULTS

The antibiotic activities of the simulated polymyxin B regimens against *A. baumannii* ATCC 19606 (Fig. 2) and 03-149.01 (Fig. 3) were determined over 336 h. With all regimens against both isolates, there was a rapid initial decline in which the reductions in the total population occurred very early in therapy, within the first 2 to 6 h of polymyxin exposure. Greater polymyxin B exposure across the first day resulted in larger maximal log reductions in bacterial counts from the baseline for both ATCC 19606 for regimens 1, 2, 3, 4, and 5 (−1.17, −1.43, −2.84, −3.40, and −1.96, respectively) and the 03-149.01 isolate (−1.25, −1.30, −2.10, −2.82, and −2.54, respectively) within the first 6 h. The rapid initial bactericidal activity that was present in the first 6 h was followed by the regrowth of the total population by 24 h for both isolates, although the degree of regrowth varied between the two isolates. For ATCC 19606, regrowth occurred within 24 h for all polymyxin B regimens, after which counts were uniformly stable up to 336 h. For 03-149.01, regrowth occurred rapidly for lower-intensity regimens (non-loading dose and loading dose) and was delayed for high-intensity regimens (front-loading, burst, and supraburst).

The polymyxin-resistant subpopulations detected over 336 h are shown in Fig. 4 for ATCC 19606 and Fig. 5 for 03-149.01. The non-loading dose regimens amplified resistant subpopulations at 24 h, resulting in resistant subpopulations that were major components of the overall population. The amplification observed within the first 24 h was driven by drug exposure, with higher-initial-exposure polymyxin regimens conferring higher levels of resistance. For resistant subpopulations, greater drug exposure also resulted in polymyxin-dependent growth: higher bacterial counts were noted on polymyxin-containing agar than on drug-free agar. This phenomenon was particularly evident when *A. baumannii* bacteria were exposed to high-intensity regimens (front-loading, burst, and supraburst) and was more pronounced in the clinical *A. baumannii* isolate 03-149.01 than in the ATCC isolate.

The population analysis profiles (PAPs) resulting from the simulated polymyxin B regimens are shown in Fig. 6 for *A. baumannii* 03-149-1. After 24 h of drug exposure in the HFIM, 03-149.01 demonstrated a polymyxin B-dependent profile, where more bacteria grew on agar containing polymyxin B than on drug-free plates. The polymyxin dependence was most evident when the high-exposure regimens (front-loading, burst, and supraburst)

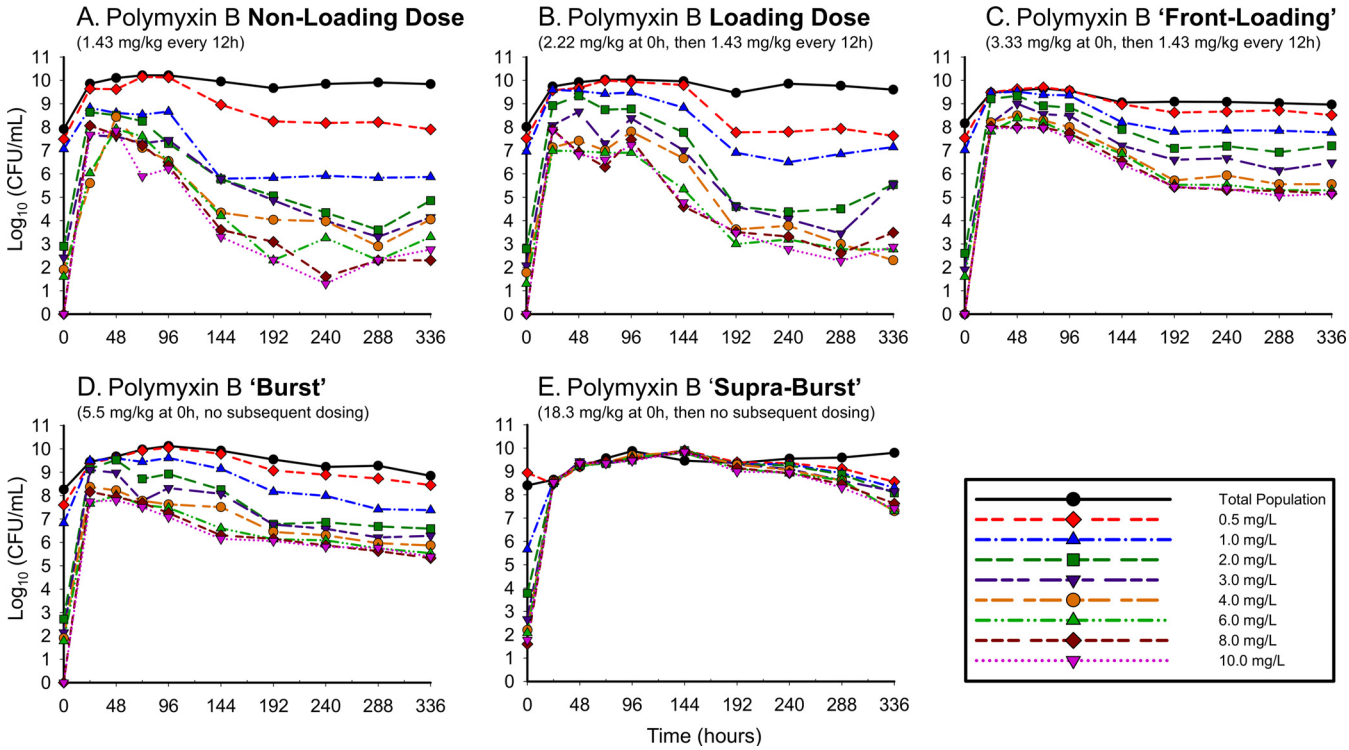

FIG 4 Complete time course of subpopulations of ATCC 19606 plated on polymyxin B-containing agar in response to polymyxin B regimens in the hollow-fiber infection model. The following regimens for polymyxin B were simulated: polymyxin B non-loading dose (1.43 mg/kg q12h, generating an $fAUC_{0-24}$ of 28.9 mg · h/liter across the first day and an $fAUC_{ss}$ of 35.9 mg · h/liter at steady state) (A); polymyxin B with loading dose (2.22 mg/kg for 1 dose followed by 1.43 mg/kg q12h staring 12 h later, $fAUC_{0-24}$ of 35.9 mg · h/liter across each day) (B); polymyxin B front-loading dose (3.33 mg/kg for 1 dose followed by 1.43 mg/kg q12h starting 12 h later, $fAUC_{0-24}$ of 48.2 mg · h/liter across the first day and an $fAUC_{ss}$ of 35.9 mg · h/liter at steady state) (C); polymyxin B burst (5.53 mg/kg for 1 dose followed by no subsequent doses, $fAUC_{0-24}$ of 60.4 mg · h/liter) (D); polymyxin B supraburst (18.4 mg/kg for 1 dose followed by no subsequent doses, $fAUC_{0-24}$ of 202.5 mg · h/liter) (E).

were plated on polymyxin B-containing agar at 24 h, at which point counts on agar plates containing 10 mg/liter polymyxin B exceeded the counts on plates which contained no polymyxin (0 mg/liter), as shown in Fig. 6A. The magnitude of dependence rose with increasing drug exposure. Front-loading, burst, and supraburst regimens displayed bacterial counts on drug-containing plates (polymyxin B drug plates of 10 mg/liter) that were 1.65, 1.84, and 2.55 $\log_{10}$ CFU/ml higher than those on drug-free agar, respectively. Although the polymyxin-dependent growth on the 48-h PAPs (Fig. 6B) was less substantial than that of the 24-h PAPs, polymyxin B-dependent growth was also observed, as the front-loading, burst, and supraburst regimens displayed 1.49, 1.32, and 1.00 $\log_{10}$ CFU/ml higher bacterial counts, respectively, on drug-containing plates (polymyxin B drug plates of 10 mg/liter) than on drug-free agar. The polymyxin dependence again was observed at 72 h (Fig. 6C); however, from 96 h to 336 h (Fig. 6D and E), bacterial counts on drug-free versus drug-containing agar were similar.

The PK/PD relationship based upon analyzing the benefit of killing and cost of resistance in the clinical isolate 03-149.01 as it relates to exposure (i.e., the regimens) is shown in Fig. 7. The log ratio area approach for 24-h bacterial killing of the total population and the 336-h resistant subpopulations growing on agar containing 3, 4, 6, 8, and 10 mg/liter of polymyxin B were well characterized by Hill-type mathematical models, with R^2 values of 0.995 and 0.998, respectively. The respective parameter estimates for the log ratio area approach for 24-h bacterial killing of the total population and the 336-h resistant subpopulations were the following: E_{max}, 2.78 and −4.72; EC_{50}, 38.5 and 34.9; and H, 10.6 and 10.2. At initial exposures (i.e., $fAUC_{0-24}$) of <38.5 mg · h/liter, increasing the polymyxin B fAUCs resulted in increased bacterial killing while gradually amplifying the development of resistant subpopulations. At the intersection point of 38.5 mg · h/liter of polymyxin exposure, the amount of bacterial killing was equivalent to the amount of resistance that was amplified. At high exposures of >38.5 mg · h/liter, the benefit of increased bacterial killing was greatly exceeded by the cost of amplification of polymyxin resistance until both phenomena plateaued at ~60 mg · h/liter.

DISCUSSION

The polymyxin antibiotics have become a last line of defense against *A. baumannii* isolates that are resistant to traditional therapies, including carbapenems (23, 24). Colistin methanesulfonate (CMS) is an inactive and inefficient prodrug that is slowly and incompletely converted to the active form of colistin (16). Polymyxin B does not suffer from these limitations, as it is administered in its active form. Administering high-intensity polymyxin B regimens during the first several hours may be highly beneficial from a bacterial killing perspective. Therefore, in the current study we utilized the HFIM to evaluate the impact of increasing the initial dose of polymyxin B against a high bacterial density of *A.*

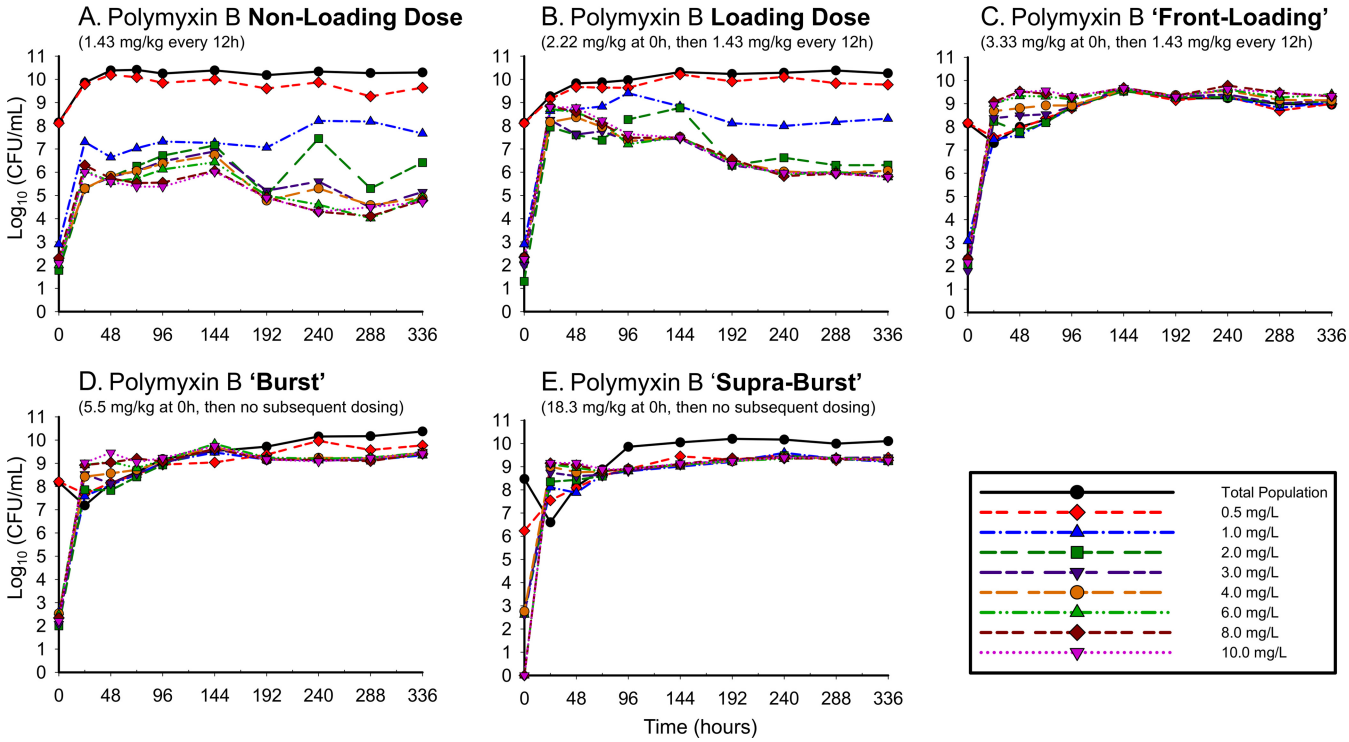

FIG 5 Complete time course of subpopulations of 03-149.01 *A. baumannii* plated on polymyxin B-containing agar in response to polymyxin B regimens in the hollow-fiber infection model. The following regimens for polymyxin B were simulated: polymyxin B non-loading dose (1.43 mg/kg q12h, generating an $fAUC_{0-24}$ of 28.9 mg · h/liter across the first day and an $fAUC_{ss}$ of 35.9 mg · h/liter at steady state) (A); polymyxin B with a loading dose (2.22 mg/kg for 1 dose followed by 1.43 mg/kg q12h starting 12 h later, $fAUC_{0-24}$ of 35.9 mg · h/liter across each day) (B); polymyxin B front-loading (3.33 mg/kg for 1 dose followed by 1.43 mg/kg starting 12 h later, $fAUC_{0-24}$ of 48.2 mg · h/liter across the first day and an $fAUC_{ss}$ of 35.9 mg · h/liter at steady state) (C); polymyxin B burst (5.53 mg/kg for 1 dose followed by no subsequent doses, $fAUC_{0-24}$ of 60.4 mg · h/liter) (D); polymyxin B supraburst (18.4 mg/kg for 1 dose followed by no subsequent doses, $fAUC_{0-24}$ of 202.5 mg · h/liter) (E).

baumannii. A number of observations emerged from the present investigation.

First, we were unable to identify a regimen of polymyxin B that eradicated *A. baumannii* at a high inoculum. It has been well established that both polymyxin B and colistin are concentration-dependent antibiotics, and the area under the curve/MIC ratio (AUC/MIC) best correlates with their efficacy (25, 26). However, the PK/PD relationship for polymyxin B as it relates to suppression of resistance is largely unknown. Specifically, it is unclear whether higher exposures of polymyxin B could overcome resistance. A number of antibiotics in the literature have demonstrated an inverted U phenomenon, whereby resistant subpopulations rose initially and then declined with increasing drug exposure until reaching a threshold that prevented resistance (27, 28). For polymyxin B, resistance amplification continued despite high *f*AUC exposures. At polymyxin exposures beyond an $fAUC_{0-24\ h}$ of 38.5 mg · h/liter, increasing the dose intensity resulted in continual escalation of resistance amplification. Somewhat to our surprise, in our proof-of-concept HFIM arms, supratherapeutic exposures of polymyxin B resulted in initial rapid bacterial killing followed by the rapid development of resistance. Therefore, driving regimens toward complete eradication may be an important endpoint for resistance suppression, and this may be possible only for polymyxin B in combination with other antibiotics.

Second, there appears to be limited utility in using polymyxin B monotherapy against high-density *A. baumannii* infections (18). In a recent multicenter study, 94 of 105 (89.5%) patients who were treated with intravenous CMS were diagnosed with bacterial pneumonia (9). In these infections, the bacterial density of the total population is remarkably high and, as a consequence, results in a higher frequency of resistant mutants (19). Zaccard et al. determined that a significant proportion of patients (26.1%) with Gram-negative pneumonia have been shown to have a bacterial burden higher than $\geq 3 \times 10^7$ in dilution-transformed colony counts of bronchoalveolar lavage fluid (17, 29). Based upon this, the bacterial inocula simulated in the HFIM were approximately 10^8 CFU/ml to mimic these clinical scenarios. Against these high bacterial densities, commonly administered clinical maintenance doses of polymyxin B resulted in stasis. Additionally, the rapid bacterial killing demonstrated on day 1 was no longer seen on days 2 to 14, where the total population was primarily comprised of cells that were completely resistant to polymyxin B. However, the development of polymyxin resistance driven by drug selective pressure may be even more difficult to observe *in vivo*. Nevertheless, 20 patients were identified with colistin-resistant *A. baumannii* which occurred almost exclusively in CMS-treated patients (13). Taken together, defining the impact of aggressive polymyxin regimens in patients with *A. baumannii* pneumonia is complex, as it also involves additional factors, such as virulence, granulocytes, and the production of chemokines and cytokines (30–32).

Third, in the present study we determined that higher drug exposures resulted in *A. baumannii* resistant subpopulations that

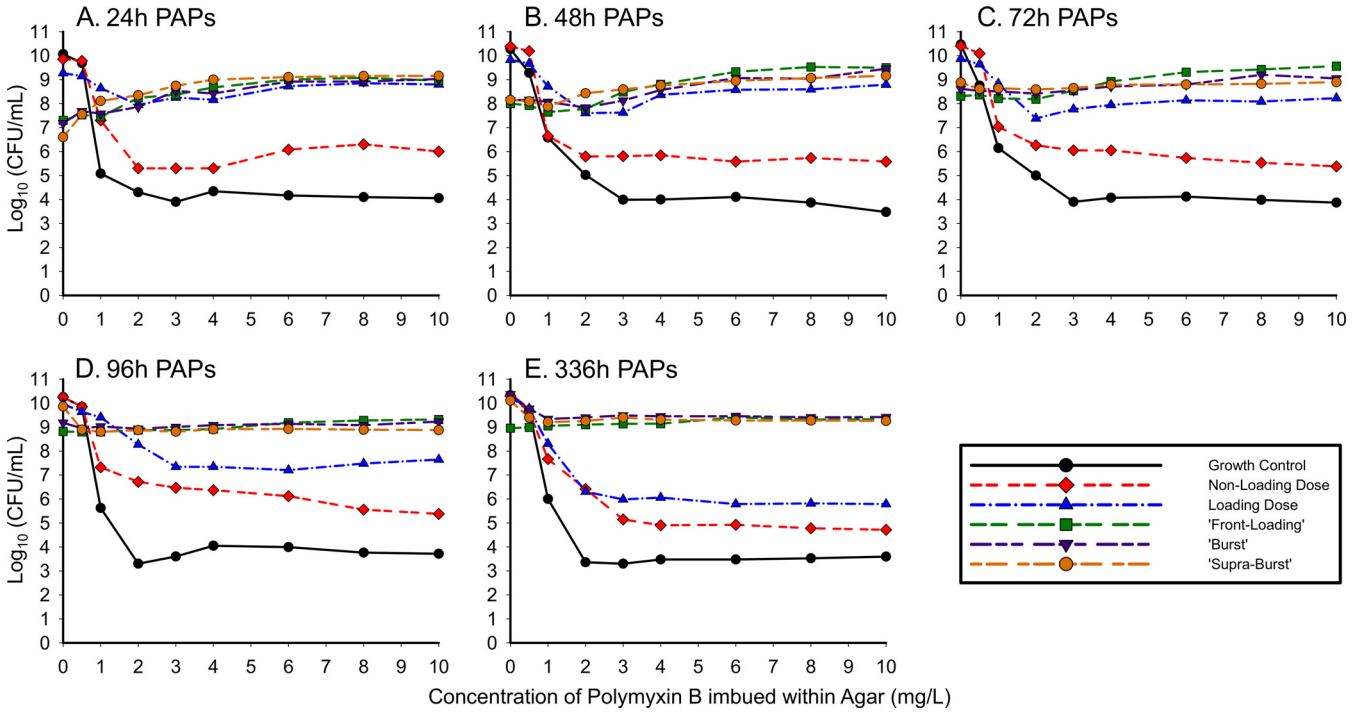

FIG 6 Real-time population analysis profiles (PAPs) for 03-149.01 comparing different dosing regimens at 24 h (A), 48 h (B), 72 h (C), 96 h (D), and 336 h (E). Samples were quantified for total population by depositing appropriately diluted bacterial samples on MHA plates. Aliquots of the diluted sample were plated on CAMHA plates containing polymyxin B at 0.5, 1, 2, 3, 4, 6, 8, or 10 mg/liter for an in-depth analysis of the time course of resistant subpopulations at the times indicated in the panels (data for 144 h, 192 h, 240 h, and 288 h are not shown).

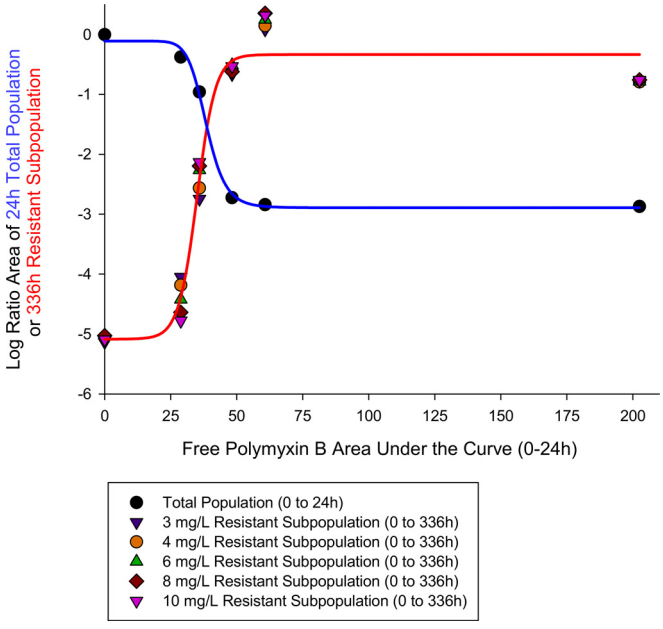

FIG 7 Cost-benefit PK/PD relationship for increasing polymyxin B exposure against 03-149.01. Benefit (blue line) was defined as the initial bacterial killing of the total population within the first 24 h. Cost (red line) was the amplification of resistant subpopulations, which was based upon real-time PAP data over 336 h that tracked *A. baumannii* growth on agar containing polymyxin B at 3, 4, 6, 8, and 10 mg/liter. The intersecting *f*AUC for the drug exposure at which the benefit of bacterial killing equaled the cost of resistance amplification was 38.5 mg · h/liter.

demonstrated a dependence on polymyxin B for growth: the bacterial counts after 24 h of exposure to polymyxin B in the HFIM were significantly higher on drug-containing agar than on drug-free agar. Alterations of lipopolysaccharide in the development of resistance have been proposed as a mechanism of colistin resistance (33–36). Qureshi et al. recently determined that lipid A modification by the addition of phosphoethanolamine was a primary mechanism for colistin resistance in colistin-resistant *A. baumannii* isolates from patients with VAP (13). Moffatt et al. previously determined that the complete loss of LPS is another mechanism contributing to colistin resistance (35, 36). Recent metabolomic studies determined that the development of resistance in a lipopolysaccharide (LPS)-deficient, polymyxin-resistant strain (19606R) was due to perturbation in specific amino acid and carbohydrate metabolites, particularly pentose phosphate pathway and tricarboxylic acid cycle intermediates (36, 37). Metabolomic and structural analyses of a polymyxin-resistant isolate, which emerged from the original 03-149-1 isolate (from the current study), identified the modification of lipid A with phosphoethanolamine (37). Taken together with the potential for evolution of resistance during polymyxin therapy in patients (13), the dose intensity and extent of killing by the first dose of polymyxin B also may play a role in driving these genomic and metabolomic changes.

Finally, it is important to note the situations in which the paradoxical effect for polymyxin B may be relevant. The paradoxical effect for polymyxin B may occur only in the context of a high bacterial density under optimal growth conditions. At lower bacterial densities, high exposure of polymyxin B has been shown to result in rapid bacterial killing and complete suppression of resis-

tance (23, 36). The pharmacodynamics of polymyxin B will also differ in patients with an intact immune system, owing to the distinct relationship between granulocyte-mediated killing and antibiotics (14). Nevertheless, the paradoxical effect for polymyxin B may also apply to other Gram-negative pathogens and other polymyxin antibiotics such as colistin when given as a standalone agent.

In conclusion, we observed a paradoxical effect whereby higher polymyxin B exposures resulted in the increased amplification of resistant, polymyxin B-dependent subpopulations. Therefore, the utility of administering polymyxin B in a high-intensity fashion may be restricted to aggressive combination regimens that combat resistance amplification. Although increasing the dose of polymyxin B in combination regimens significantly improves bacterial killing, the probability of nephrotoxicity increases as well (37–39). Now, the challenge is to define optimal pharmaco- and toxicodynamically driven regimens for polymyxin B combinations which result in the greatest bacterial killing and suppression of resistance and the least potential toxicity.

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