To compare the antipseudomonal efficacy of doripenem and imipenem as well as their abilities to restrict the enrichment of resistant *Pseudomonas aeruginosa*, multiple-dosing regimens of each drug were simulated at comparable values of the cumulative percentages of a 24-h period that the drug concentration exceeds the MIC under steady-state pharmacokinetic conditions ($T_{\geq \text{MIC}}$) and ratios of the 24-hour area under the curve (AUC$_{24}$) to the MIC. Three clinical isolates of ciprofloxacin-resistant *P. aeruginosa* (MIC of doripenem, 1 µg/ml; MICs of imipenem, 1, 2, and 2 µg/ml) were exposed to thrice-daily doripenem or imipenem for 3 days at AUC$_{24}$/MIC ratios of from 50 to 170 h (doripenem) and from 30 to 140 h (imipenem). The antimicrobial effects for susceptible and resistant subpopulations of bacteria were expressed by the areas between control growth and time-kill curves ($I_k$) and areas under the bacterial mutant concentration curves (AUBCM), respectively. With each antibiotic, the $I_k$ and AUBCM versus log AUC$_{24}$/MIC relationships were bacterial strain independent. At similar AUC$_{24}$/MIC ratios, doripenem was slightly less efficient than imipenem against susceptible and resistant subpopulations of bacteria. However, doripenem appeared to be somewhat more efficient than imipenem at clinically achievable AUC$_{24}$s related to the means of the MICs for the three studied strains and had higher antimutant potentials for two of the three strains.

**Pseudomonas aeruginosa** remains an important cause of nosocomial infections of the lung, urinary tract, and bloodstream, especially within intensive care units (13, 19). This organism is particularly problematic for critically ill, neutropenic, immunocompromised, and mechanically ventilated patients (21). Over the past decade, fluoroquinolone resistance among these organisms has increased, often requiring other antibiotics for successful treatment (20). Recent studies have suggested that beta-lactam monotherapy for serious pseudomonal infections is probably as effective as combination therapy, even though *in vitro* synergism can sometimes be demonstrated with the combinations (4, 15).

Doripenem is a new carbapenem antibiotic active against *Pseudomonas aeruginosa*. The pharmacodynamics of doripenem have been studied *in vitro* using a murine thigh model of infection with 10 Gram-negative organisms (1) and a rabbit model of pseudomonal pneumonia (3). A species-independent relationship between changes in the logarithm of the number of CFU of Gram-negative organisms per thigh and the cumulative percentages of a 24-h period that the drug concentration exceeds the MIC under steady-state pharmacokinetic conditions ($T_{\geq \text{MIC}}$) was established in the former study, and similar efficacies of doripenem, imipenem, and meropenem were reported in the latter. $T_{\geq \text{MIC}}$ relationships of doripenem and imipenem antipseudomonal effects were also observed with each of three isogenic strains in an *in vitro* study using a dynamic model (14). Because the simulated ranges of the $T_{\geq \text{MIC}}$ and the ratios of carbapenem dose (D) to the MIC overlapped only minimally, the antipseudomonal effects of doripenem and imipenem could not be compared under identical conditions. To make possible such a comparison, we studied the pharmacodynamics of doripenem and imipenem with ciprofloxacin-resistant strains of *P. aeruginosa* while simulating the pharmacokinetics at comparable $T_{\geq \text{MIC}}$s and ratios of the 24-hour area under the curve (AUC$_{24}$) to the MIC.

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for the doripenem plates ranged from 1 to 32 μg/ml, and for imipenem they ranged from 128 to 2,048 μg/ml. The inoculated plates were incubated for up to 144 h at 36°C and visually screened for growth. The MPC was taken as the lowest antibiotic concentration that completely inhibited growth. The MPCs for all strains were 8 μg/ml for doripenem and 1,024 μg/ml for imipenem.

In vitro dynamic model and simulated pharmacokinetic profiles. A previously described dynamic model (2) was used in the study. Briefly, this two-compartment model consists of the central compartment and three bioreactors, artificial chambers (Fibercell Systems Inc., Frederick, MD) connected in series, which represent the peripheral compartments. For all experiments, the bacterial inoculum was prepared from previously frozen inocula by thawing, diluting with MHB/S, and then incubating at 36°C to bring the organisms into growth phase.

This mixture was then inoculated via an entry port into each peripheral compartment, which also contained MHB/S, and incubated until a density of approximately 10^8 CFU/ml was achieved, at which time the antibiotic was introduced into the central compartment (time zero). Given a 20-ml volume of the peripheral compartment, the total number of organisms in the starting inoculum reached approximately 2 × 10^9 CFU.

**FIG 1** AUBC_{8h} analysis of population data (P. aeruginosa 8996 exposed to doripenem at AUC_{8h}/MIC of 50 h). AUBC_{8h} are indicated by shaded areas.

**FIG 2** Simulated pharmacokinetics [(AUC_{24}/MIC)/(C_{max}/MIC)/T_{>MIC}] of doripenem and imipenem and time courses of susceptible (0×MIC) and resistant (2×, 4×, 8×, and 16×MIC) subpopulations of P. aeruginosa 8997 exposed to the antibiotics. Antibiotic dosing is indicated by arrows.
Control experiments without antimicrobial agent were performed to characterize growth kinetics.

A series of monoeponential profiles that mimic thrice-daily administration (60-min infusion) of doripenem and imipenem (half-life of each, 1 h) (18, 22) was simulated for 3 consecutive days.

Simulated AUC_{24}/MIC ratios varied from 60 to 180 h for doripenem and from 30 to 120 h for imipenem. These ranges incorporate the clinically attainable AUC_{24}/MIC ratios that can be achieved in humans infected by P. aeruginosa isolates with MICs equal to the geometric mean of MICs for the three studied strains after thrice-daily administration of 0.5 g doripenem and imipenem.

As the antimicrobial effect depends on antibiotic concentrations in the peripheral compartments (where the organisms come into contact with antibiotic), peripheral compartments were sampled to determine antibiotic concentrations using an agar well diffusion bioassay. Difco antibiotic plates were also inoculated at 0, 24, 48, 72, and (if needed) 96 h onto plates containing 2\(\times\), 4\(\times\), 8\(\times\), and 16\(\times\) MIC of the antibiotic being tested during the experiment. After overnight incubation at 36°C, the resulting bacterial colonies were counted, and the numbers of CFU/ml were calculated. The detection limit was 167 CFU/ml. The duration of the experiments was at least 72 h.

Based on time-kill data, the intensity of the antimicrobial effect (the area between control growth and time-kill curves [AUC_{24}/MIC]) from time zero to the time after the last antibiotic dose at which the number of antibiotic-exposed bacteria reached 10^9 CFU/ml was determined.

To delineate AUC_{24}/MIC relationships with resistance, areas under the bacterial mutant concentration curves (AUC_{24}) (9) were determined for subpopulations resistant to 2\(\times\), 4\(\times\), 8\(\times\), and 16\(\times\) MIC of antibiotic from the beginning of treatment to 72 h (Fig. 1). Changes in susceptibility of P. aeruginosa were examined by MIC determinations using bacteria from drug-free plates inoculated at 0, 24, 48, 72, and (if applicable) 96 h during each experiment. Stability of the observed resistance was determined daily by consecutive passaging of P. aeruginosa on antibiotic-free agar plates for 5 consecutive days.

To reconstruct AUC_{24}/MIC-response and maximum concentration of drug in plasma (C_{\text{max}}/MIC)-response relationships with doripenem from the above-mentioned in vivo study (1), T_{MIC} curves of the 24-hour change in the log number of CFU per thigh (Alog \(N_{24}\)) in mice infected with Escherichia coli, Enterobacter cloaceae, and P. aeruginosa were scanned and digitized (Graphula 3 software, version 2.10). Data reported with Klebsiella pneumoniae could not be properly distinguished and, therefore, were excluded from our analysis. Because of pronounced scattering of the points, log AUC_{24}/MIC, log C_{\text{max}}/MIC, and T_{MIC} versus Alog \(N_{24}\) data sets were fitted by a linear regression rather than a sigmoid equation. Scanned and digitized time-kill data that were reported from the in vitro study (14) were used to combine data obtained with three organisms and to reconstruct the respective T_{MIC} and log D/MIC relationships of log \(N_{24}\).

RESULTS

Carbapenem pharmacodynamics with susceptible P. aeruginosa. Simulated pharmacokinetic profiles and killing kinetics of one of the studied strains (P. aeruginosa 8997) exposed to doripenem and imipenem are shown in Fig. 2. As seen in the figure, an approximately 1.5-fold range of the T_{MIC} for doripenem was provided by the 3.4-fold ranges of the AUC_{24}/MIC and C_{\text{max}}/MIC ratios. Ratios with imipenem, a 1.6-fold range of the T_{MIC} was provided by the 4.5- to 4.7-fold ranges of the AUC_{24}/MIC and C_{\text{max}}/MIC ratios. The determined antibiotic concentrations were close to the designed values. With doripenem, the observed AUC_{24}/MIC ratios (50, 90, and 170 h) closely approximated the targeted AUC_{24}/MIC ratios (60, 90, and 180 h, respectively). Similar concordance between the observed and designed concentrations was observed in simulations with imipenem (30, 70, and 140 h versus 30, 60, and 120 h, respectively).

As seen in Fig. 2, with an increase in the simulated AUC_{24}/MIC, C_{\text{max}}/MIC, and T_{MIC}, the rate of killing of doripenem-exposed P. aeruginosa increased weakly: at the highest AUC_{24}/MIC, the time required to kill 99% of the bacteria (T_{MIC}) was only 2-fold shorter than that at the lowest AUC_{24}/MIC ratio. Unlike T_{MIC}, the extent of bacterial killing that is reflected by the minimum number of bacteria (N_{\text{min}}) decreased systematically with an increase in the simulated AUC_{24}/MIC, C_{\text{max}}/MIC, and T_{MIC}. At the highest AUC_{24}/MIC ratio, the N_{\text{min}} was more than 4 orders lower than that at the lowest AUC_{24}/MIC ratio. The concentration-dependent—more specifically, the AUC_{24}/MIC-dependent—lowering...
of the $N_{\text{min}}$ was accompanied by later bacterial regrowth. At the highest AUC$_{24}$/MIC ratio, the regrowth was observed after the end of treatment, whereas at the lowest AUC$_{24}$/MIC ratio, it had already occurred on the third day of treatment.

Similar patterns were inherent in killing kinetics of *P. aeruginosa* 8997 exposed to imipenem. Like doripenem, bacterial regrowth followed the concentration-dependent reduction in bacterial counts over the entire range of the simulated AUC$_{24}$/MIC ratios of imipenem. Again, $T_{99\%}$ appeared to be only minimally sensitive to the AUC$_{24}$/MIC, $C_{\text{max}}$/MIC, and $T_{\text{MIC}}$, whereas the $N_{\text{min}}$ was much smaller and the time to regrowth was longer at the highest AUC$_{24}$/MIC ratio than at the lowest AUC$_{24}$/MIC ratio.

Time-kill curves obtained with *P. aeruginosa* 8996 and *P. aeruginosa* 14051 exposed to doripenem and imipenem (data not shown) were similar to those observed with *P. aeruginosa* 8997.

When expressed by the $I_E$ parameter, the effects of doripenem and imipenem on *P. aeruginosa* were dependent on the AUC$_{24}$/MIC, $C_{\text{max}}$/MIC, and $T_{\text{MIC}}$ in a strain-independent manner (Fig. 3). There were strong correlations between the $I_E$ and each of the three predictors of the antibacterial effect. For this reason, only the AUC$_{24}$/MIC relationships of the $I_E$ were used in further comparisons of doripenem with imipenem.

With each organism exposed to doripenem, the $I_E$s observed at comparable AUC$_{24}$/MIC ratios were lower than those for imipenem (Fig. 4, top). However, at the clinically achievable AUC$_{24}$/MIC ratios (130 h versus 65 h for *P. aeruginosa* 8996 exposed to doripenem and imipenem, respectively, and 130 h for *P. aeruginosa* 14051 exposed to both antibiotics), the predicted antipseudomonal effects were similar, whereas the effect of doripenem on *P. aeruginosa* 8997 was greater than that of imipenem (AUC$_{24}$/MIC ratios, 130 and 65 h, respectively).

Combined data from all the three strains also exhibit carbapenem-specific AUC$_{24}$/MIC-response plots (Fig. 5, left): the difference between the respective regressions was statistically significant ($P < 0.05$), in terms of both the slope and the intercept. Because the doripenem plot is under the imipenem plot over the studied AUC$_{24}$/MIC ranges, a higher AUC$_{24}$/MIC ratio of doripenem is needed to provide the same antipseudomonal effect as imipenem. However, at clinically achievable AUC$_{24}$ related to the geometric means of the MICs for the three studied strains (1 $\mu$g/ml of doripenem and 1.6 $\mu$g/ml of imipenem), doripenem appeared to be slightly more efficient than imipenem, although this difference should not be considered substantial due to the pronounced scattering of the points.
Carbapenem pharmacodynamics with resistant subpopulations of *P. aeruginosa*. As seen in Fig. 2, at the lowest and intermediate AUC24/MIC ratios (50 and 90 h with doripenem and 30 and 70 h with imipenem), killing of carbapenem-susceptible subpopulations of *P. aeruginosa* 8997 was accompanied by the enrichment of resistant subpopulations. These subpopulations were enriched earlier at the lowest AUC24/MIC ratios than the intermediate AUC24/MIC ratios. However, by the end of treatment, subpopulations resistant to 2× and 4× MIC of doripenem (AUC24/MIC, 50 h) and to 2×, 4×, and 8× MIC of doripenem (AUC24/MIC, 90 h) and subpopulations resistant to 2×, 4×, 8×, and 16× MIC of imipenem (AUC24/MIC ratios, 30 and 70 h) completely replaced carbapenem-susceptible organisms. In contrast, at the highest AUC24/MIC ratios (170 and 90 h with doripenem and imipenem, respectively), resistant subpopulations were not amplified during treatment. Time courses observed with resistant subpopulations of *P. aeruginosa* 8996 and *P. aeruginosa* 14051 exposed to doripenem and imipenem (data not shown) were similar to those observed with *P. aeruginosa* 8997.

As seen in Fig. 6, a pronounced enrichment of resistant subpopulations occurred immediately after cessation of treatment of *P. aeruginosa* 8997 with imipenem (AUC24/MIC, 140 h) but not doripenem (AUC24/MIC, 170 h). The same difference was observed after the treatment of *P. aeruginosa* 8996 (Fig. 6): the size of subpopulations resistant to 2×, 4×, 8×, and 16× MIC of imipenem was 4 to 7 orders higher than that of doripenem-resistant subpopulations. The amplification of imipenem-resistant subpopulations was accompanied by 16-fold (*P. aeruginosa* 8996) and 8-fold (*P. aeruginosa* 8997) elevations in the MICs (Fig. 7). The observed elevations in the MICs were stable after five passages on antibiotic-free agar plates. In contrast to imipenem, there was no loss in susceptibility of *P. aeruginosa* 8996 and 8997 treated with doripenem. Unlike *P. aeruginosa* 8996 and 8997, an increase in the size of resistant *P. aeruginosa* 14051 subpopulations occurred after treatment with both doripenem and imipenem.

Based on time courses of carbapenem-resistant subpopulations, the AUBCMs were plotted against the AUC24/MIC ratio for each organism (Fig. 4, bottom). As seen in the figure, at clinically achievable AUC24/MIC ratios, doripenem exhibits lower AUBCMs, i.e., greater antimutant effects, than imipenem against *P. aeruginosa* 8996 and 8997, which is reversed with *P. aeruginosa* 14051. The AUC24/MIC relationships of the AUBCMs determined with resistant subpopulations of the three studied strains exposed to doripenem and imipenem are shown on the right of Fig. 5.

Although AUC24/MIC plots of the AUBCMs were not superimposed for doripenem and imipenem, the difference between the respective regressions was statistically insignificant (*P = 0.05*). With doripenem, the predicted AUBCM was slightly smaller (greater antimutant effect) than that with imipenem.

**DISCUSSION**

Designed to ascertain concentration-response relationships with two carbapenems, this study predicts similar or slightly greater effects of doripenem than imipenem on susceptible and resistant subpopulations of *P. aeruginosa* exposed to clinically achievable AUC24/MIC ratios. These findings are consistent with clinical reports of the similar efficacies of doripenem and imipenem (5) and the somewhat higher efficacy of doripenem in ventilator-associated pneumonia (16, 17). A more pronounced enrichment of resistant subpopulations observed in our study after (but not during) treatment with imipenem can be attributed to its greater MPC (1,024 μg/ml) compared to that of doripenem (8 μg/ml).

Contrary to the widespread idea that carbapenem effects are dependent on *T*ₘᵢᴄ but not AUC₂₄/MIC and Cₘₐₓ/MIC, all three predictors exhibited similar correlations with the antipseudomonal effect (Fig. 3). The same conclusion can be drawn from recently reported data (14) that were originally interpreted using *T*ₘᵢᴄ only. Despite the fact that the authors avoided any mention of AUC₂₄/MIC or Cₘₐₓ/MIC, due to the similar pharmacokinetics of doripenem and imipenem (18, 22), the respective AUC₂₄/MIC relationships of the effect can be represented by its relationships to 

![FIG 6](http://aac.asm.org/)

**FIG 6** Resistant subpopulations of *P. aeruginosa* after exposure to doripenem (AUC₂₄/MIC, 170 h) and imipenem (AUC₂₄/MIC, 140 h). Black bars, doripenem; gray bars, imipenem.

![FIG 7](http://aac.asm.org/)

**FIG 7** Changes in susceptibility of *P. aeruginosa* exposed to doripenem (AUC₂₄/MIC, 170 h) and imipenem (AUC₂₄/MIC, 140 h); ratio of the MIC determined 96 h after the start of treatment (MIC₉₆h) to the initial MIC (MIC₀). The descriptions of the symbols are the same as those in the legend to Fig. 6.
the MIC-related daily dose that corresponds to the clinical AUC$_{24}$/MIC for the unbound antibiotic (D/MIC). As seen in Fig. 8, killing of P. aeruginosa exposed to each carbapenem correlates with the D/MIC ratio (and, therefore, with AUC$_{24}$/MIC) even better than with T$_{>}$MIC. Furthermore, according to our analysis of reported in vivo data (1), the abilities of AUC$_{24}$/MIC, C$_{max}$/MIC, and T$_{>}$MIC to predict doripenem effects in a neutropenic murine thigh infection model are also similar (r$^2$ = 0.77, 0.77, and 0.67, respectively). This analysis once again raises questions about the advisability of searching for the “best” predictor of the antibacterial effect among highly covarying variables. Attempts to contrast equally good (or equally bad) predictors have been criticized previously (7, 10). Moreover, these attempts were often incorrect: erroneous conclusions about the advantages of one predictor over another resulted from the use of inconsistent functions relating the effect to its predictors, inappropriate combining of data obtained with pharmacokinetically different antibiotics, etc. Methodological pitfalls in analysis of the predictor-response relationships have been discussed in detail elsewhere (12).

Overall, at clinically achievable AUC$_{24}$/MIC ratios, doripenem was slightly more efficient than imipenem and prevented mutant selection somewhat better for two of the three tested strains.

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REFERENCES