Evaluation of Meropenem Regimens Suppressing Emergence of Resistance in *Acinetobacter baumannii* with Human Simulated Exposure in an *In Vitro* Intravenous-Infusion Hollow-Fiber Infection Model

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The emergence of resistance to carbapenems in *Pseudomonas aeruginosa* can be suppressed by optimizing the administration of meropenem. However, whether the same is true for *Acinetobacter baumannii* is not fully understood. We assessed the bactericidal activity of meropenem and its potency to suppress the emergence of resistance in *A. baumannii* with human simulated exposure in an *in vitro* intravenous-infusion hollow-fiber infection model (HFIM). Two clinical strains of carbapenem-susceptible multidrug-resistant *A. baumannii* (CS-MDRAB), CSRA24 and CSRA91, were used, and their MICs and mutant prevention concentrations (MPCs) were determined. Six meropenem dosage regimens (0.5, 1.0, or 2.0 g given every 8 h [q8h] with a 0.5-h or 3-h infusion for seven consecutive days) were simulated and then evaluated in the HFIM. Both the total population and resistant subpopulations of the two strains were quantified. Drug concentrations were measured by high-performance liquid chromatography. All dosage regimens, except for the lowest dosage (0.5 g for both the 0.5-h and 3-h infusions), showed 3-log CFU/ml bacterial killing. Dosage regimens of 2.0 g with 0.5-h and 3-h infusions exhibited an obvious bactericidal effect and suppressed resistance. Selective amplification of subpopulations with reduced susceptibility to meropenem was suppressed with a percentage of the dosage interval in which meropenem concentrations exceeded the MPC ($T > \text{MPC}$) of $\geq 20\%$ or with a ratio of $T > \text{MPC}$ to the percentage of the dosage interval in which drug concentrations are within the mutant selection window of ≥ 0.25 . Our *in vitro* data support the use of a high dosage of meropenem (2.0 g q8h) for the treatment of severe infection caused by CS-MDRAB.

Acinetobacter baumannii is classified under the *Acinetobacter* genus and comprises strictly aerobic, Gram-negative, non-motile, non-lactose-fermenting, oxidase-negative, catalase-positive coccobacilli (1). *A. baumannii* is an opportunistic pathogen associated with severe nosocomial infections and often exhibits multidrug resistance to most of the currently available antibiotic agents (1–3). Carbapenems, which are a class of β -lactam antibiotics including imipenem and meropenem, have been recommended as the last resort for the treatment of infection with multidrug-resistant *A. baumannii* (MDRAB) (4). However, a substantial increase in the prevalence of carbapenem-resistant *A. baumannii* strains has been documented during the last few years, which has become a serious problem that threatens our therapeutic armamentarium for *A. baumannii* infection (5–7). However, the development process for new drugs against *A. baumannii* infection is very slow, which indicates that overcoming multidrug resistance in *A. baumannii* cannot rely solely on the development and availability of new drugs; it is imperative that currently available antimicrobial agents be preserved as the mainstream treatment to fight against the emergence of resistance in *A. baumannii* by optimizing the use of these agents.

It has been shown that suboptimal dosing of antibiotics provides a selective pressure on bacteria and facilitates the emergence of resistance resulting from bacterial mutation under selective pressure of antibiotics (8, 9). In 1998, Nunez et al. reported a meningitis case caused by an *Acinetobacter* strain that developed resistance to meropenem during treatment with this agent (10). Several studies have identified the administration of carbapenems as an independent risk factor for the emergence of carbapenem-

resistant MDRAB in patients (6, 11–15). It has also been confirmed that emergence of resistance in *Pseudomonas aeruginosa* can be suppressed by optimizing the administration of meropenem (16, 17). However, whether this phenomenon also applies to *A. baumannii* is not fully understood. The aim of the present study was to determine whether *in vitro*-simulated pharmacodynamic exposure to different meropenem dosage regimens is able to optimize bactericidal activity and suppress the emergence of resistance in carbapenem-susceptible MDRAB (CS-MDRAB) strains.

MATERIALS AND METHODS

Antimicrobial agents. Meropenem reference substance, obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), was used for susceptibility testing and as the standard for measurement of meropenem by high-performance liquid chromatography (HPLC), as described below. The drug was dissolved and diluted to the desired concentrations with sterilized water for injection (Shijiazhuang No. 4 Pharmaceutical Company, Shijiazhuang, Hebei, China) prior to use. In addition, meropenem powder, kindly provided by SumitomoPharma Co., Ltd. (Suzhou, Jiangsu, China), was used for phar-

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macokinetic/pharmacodynamic (PK/PD) experiments in the hollow-fiber infection model (HFIM).

Microorganisms. Two clinical strains of CS-MDRAB, CSRA24 and CSRA91, which we isolated from two unrelated patients with pulmonary infection, were used in this study. These two strains were tested to be multidrug resistant but carbapenem susceptible by routine antimicrobial susceptibility testing (Vitek 2 automated antimicrobial susceptibility testing system; bioMérieux). Briefly, CSRA24 and CSRA91 were both resistant to mezlocillin, ampicillin-sulbactam, piperacillin-tazobactam, ceftioaxone, ceftazidime, cefepime, cefotaxime, gentamicin, and tobramycin. However, CSRA91 was also resistant to ciprofloxacin and levofloxacin, whereas CSRA24 was susceptible to ciprofloxacin and levofloxacin. In addition, a *P. aeruginosa* strain, ATCC 27853, was used as a reference strain for quality control for susceptibility testing. In order to ensure the parallelism of experiments, all strains were dispensed into airtight vials in the form of a freeze-dried powder and stored at -70°C . Prior to each experiment, the strains were subcultured on Columbia plates with 5% sheep blood (bioMérieux, Shanghai, China) and incubated at 35°C for 24 h.

In vitro susceptibility testing. Susceptibility testing was performed in triplicate by the agar dilution method according to the recommendations of the Clinical and Laboratory Standards Institute (18). Serial 2-fold dilutions of meropenem (0.125 to 64 $\mu\text{g}/\text{ml}$) were prepared and incorporated into designated Mueller-Hinton agar (MHA; Oxoid, Ltd., Hampshire, England) plates. The final CFU count of the bacterial inoculum on each plate was approximately 5×10^4 CFU. The MIC was defined as the lowest concentration of the drug that resulted in no visible growth on the agar after 24 h of incubation at 35°C in a biochemical incubator.

In addition, the mutant prevention concentration (MPC) was also determined, as described previously (19). Briefly, the tested strains were cultured in Ca–Mueller-Hinton broth (MHB; Oxoid, Ltd., Hampshire, England) and incubated at 35°C for 24 h. After centrifugation at $4,000 \times g$ for 10 min, the strains were resuspended in MHB to yield a concentration of 3×10^{10} CFU/ml. Next, each of the MHA plates with one of the designated meropenem concentrations was inoculated with 1×10^{10} CFU of the strains. The inoculated plates were incubated for 72 h at 35°C in a biochemical incubator and screened visually for growth. MPC was defined as the lowest meropenem concentration that completely inhibited growth of the bacteria (20). Determination of the MPC was carried out in duplicate and was repeated two times on separate days.

Hollow-fiber infection model. The HFIM, which was previously used and described in detail (21), was modified in the present study to simulate the PK/PD of meropenem. First, an intravenous-infusion model, instead of an injection model, was applied to simulate the rising phase of the concentration-time curve. Second, reverse external circulation of the extracapillary compartment with a low flow rate was added to assess the PD of meropenem accurately. Third, the peristaltic pumps were controlled by computer software to conveniently and precisely simulate the concentration-time curve under different meropenem regimens. An F4HPS capillary dialyzer (Fresenius Medical Care [Shanghai] Co., Ltd., Shanghai, China) was used as the hollow-fiber cartridge for the HFIM. The effective surface area of the hollow-fiber systems was 0.8 m^2 . A diagram of the HFIM is shown in Fig. 1.

MHB containing the designated dose of meropenem was infused from the drug reservoir into the central reservoir by a peristaltic pump (Leadfluid BT-101L; Baoding Leadfluid Technology Co., Ltd., Baoding, China) from time zero to 3 h. Simultaneously, the medium in the central reservoir was pumped into the waste reservoir by another peristaltic pump. After 3 h, the pump controlling the drug reservoir was switched off, and the drug-free medium was infused from the diluent reservoir into the central reservoir by a third peristaltic pump. At 8 h, the peristaltic pump controlling the diluent reservoir was stopped, and the dosing process was repeated for another administration period of 7 days. The peristaltic pumps mentioned above were controlled by Labcomsoft 3.0 computer software (Baoding Leadfluid Technology Co., Ltd.) with the same designated flow rate. The 0.5-h infusion model for the designated dose of meropenem was

FIG 1 Diagram of the hollow-fiber infection model.

carried out in the same fashion, except that the infusion time was 0.5 h instead of 3 h. MHB medium without meropenem was used as a control.

The bacterial suspension (approximately 70 ml) was injected and confined in the extracapillary compartment of the hollow-fiber cartridge, and nutrients and meropenem in the broth were able to be exchanged between the extracapillary and inner capillary compartments.

Simulated PK profiles. Monoexponential profiles that mimicked three-times-daily administration (0.5-h or 3.0-h infusion) of meropenem with an elimination half-life ($t_{1/2}$) of approximately 1.0 h were simulated as previously described (22, 23). Six meropenem dosage regimens (0.5, 1.0, or 2.0 g given every 8 h [q8h] with a 0.5-h or 3-h infusion for seven consecutive days) were simulated in the HFIM, as described above. The simulated single-dose peak concentrations ($C_{\max}$) were 22.76, 45.34, and 90.68 $\mu\text{g}/\text{ml}$ for regimens of 0.5, 1.0, and 2.0 g with a 0.5-h infusion, respectively, and 9.20, 18.40, and 36.80 $\mu\text{g}/\text{ml}$ for regimens of 0.5, 1.0, and 2.0 g with a 3-h infusion, respectively.

Experimental setup. The freeze-dried powder of the strains was subcultured on Columbia plates with 5% sheep blood (bioMérieux, Shanghai, China) at 35°C for 24 h. The bacterial suspension was prepared by taking one freshly grown medium-sized colony, placing it into MHB, and incubating it further at 35°C until the bacteria reached the late log phase of growth (10^8 CFU/ml). In order to simulate the bacterial load of severe infection and detect the resistant mutant subpopulations at baseline, a bacterial suspension of 10^8 CFU/ml was then injected into the extracapillary compartment of the hollow-fiber cartridge and incubated continually at 37°C until the bacteria reached a concentration of approximately 10^9 CFU/ml (8, 24). The six meropenem regimens described above and a control (medium without meropenem) were investigated.

PK/PD validation and microbiological response. For the regimens with a 0.5-h infusion, samples (1.0 ml) were collected from the central reservoir of the HFIM before each dosing and at 0.167 h (10 min), 0.33 h (20 min), 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, and 8 h after the beginning of the last dosing. For the regimens with a 3-h infusion, samples were collected before each dosing and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, and 8 h after the beginning of the last dosing. The meropenem concentrations in these samples were assayed by HPLC, as described below. All drug samples were dispensed into 2.0-ml capped polypropylene tubes and stored at -70°C until analysis. The concentration-time profiles were modeled by fitting a one-compartment linear model with DAS version 3.0 (BioGuider Co., Shanghai, China). PK/PD parameters, including the ratio of the minimum concentration to MIC ($C_{\min}/\text{MIC}$ ratio), the percentage of the dos-

FIG 2 Mathematically simulated concentration-time curves of various meropenem regimens. (A) Regimens with 0.5- and 3-h infusions for strain CSRA24. (B) Regimens with 0.5- and 3-h infusions for strain CSRA91.

age interval in which the drug concentrations exceeded the MIC (% $T > \text{MIC}$), the percentage of the dosage interval in which drug concentrations exceeded the MPC (% $T > \text{MPC}$), the percentage of the dosage interval in which drug concentrations were within the mutant selection window (% T_{MSW}), and the ratio of $T > \text{MPC}$ to T_{MSW} ($T > \text{MPC}/T_{\text{MSW}}$ ratio) were calculated according to the steady-state concentration-time curves.

Serial bacterial samples were obtained from the extracapillary compartment of the hollow-fiber cartridge at 0, 6, 12, 18, 24, 48, 72, 96, 120, 144, and 168 h. The samples were cultured to quantitatively assess the effects of the various drug regimens on the total bacterial population and the selection of bacterial subpopulations with reduced susceptibility, as described below. Total bacterial populations were quantified by inoculating, in triplicate, the 10-fold serially diluted samples (100 μl) onto drug-free MHA plates. Subpopulations with reduced susceptibility were quantified by inoculating, in triplicate, the 10-fold serially diluted samples (100 μl) onto MHA plates containing meropenem at $3 \times \text{MIC}$. The plates were incubated at 35°C for 24 h (total population) or 72 h (subpopulations with reduced susceptibility) according to previous studies (16, 17). The bacterial colonies from each plate were counted by using an automatic colony counter (Hangzhou Shineso Co., Ltd., Zhejiang, China). Mutation was defined as occurring when any colony was present on the plates containing the drug at $3 \times \text{MIC}$. Subsequently, the mutation frequency (MF) was calculated based on the following formula: $\text{MF} = \text{bacterial counts on plates containing the drug at } 3 \times \text{MIC} / \text{bacterial counts on drug-free plates}$. Enrichment of mutant strains was defined as occurring when the lower end of the 95% confidence interval (CI) of the bacterial colonies on the plates containing the drug at $3 \times \text{MIC}$ was at least the upper end of the 95% CI of the colonies in the control at the same point. Three colonies were randomly picked from the drug-containing plates and tested for MICs of meropenem to confirm the emergence of resistance. The limit of this detection method was 10^1 CFU/ml.

HPLC. The drug concentrations of the collected samples were analyzed by HPLC (LC-20ATvp; Shimadzu, Japan) with an ODS (octadecyl silane) C_{18} column (250 mm by 4.6 mm, 5 μm) (Spursil; Dikma Technologies, Inc., Beijing, China). The mobile phase was a mixed solution of acetonitrile-methanol (10:3 [vol/vol] ratio of acetonitrile to methanol) and 0.05 mol/liter sodium dihydrogen phosphate at a ratio of 11:89 (vol/vol) (adjusted to pH 5.0 by phosphoric acid). The flow rate was 0.8 ml/min, and the detection wavelength was 298 nm. A total of 20 μl of sample was injected onto the HPLC instrument after being filtered by using a 0.22- μm polysulfone membrane. The calibration curve was linear over a range from 0.18 to 181.17 $\mu\text{g}/\text{ml}$ ($y = 6,498.23x + 5,581.00$ [$R^2 = 0.9997$], where y is the peak area of meropenem and x is the concentration of

meropenem). The precision in intra- and interday assays was between 2.01% and 4.01%.

Statistical analysis. Concentration-time curves for the six dosing regimens were simulated by DAS3.0 software (BioGuider Co., Shanghai, China). Numerical data are reported as means $\pm$ standard deviations (SD). Kaplan-Meier survival curves, with the Breslow-Gehan test, were performed with SPSS 13.0 for comparison of the bacterial killing effects (represented by the survival of bacteria at different time points) of 2.0 g q8h with a 0.5-h infusion and 2.0 g q8h with a 3-h infusion during the whole experiment. The significance level was set at a P value of <0.05 .

RESULTS

MICs and MPCs. The meropenem MICs for *A. baumannii* strains CSRA24 and CSRA91 were 2.0 $\mu\text{g}/\text{ml}$ and 0.5 $\mu\text{g}/\text{ml}$, respectively. The MIC was 0.5 $\mu\text{g}/\text{ml}$ for *P. aeruginosa* strain ATCC 27853. The meropenem MPC value was 28.8 $\mu\text{g}/\text{ml}$ for both CSRA24 and CSRA91.

Simulated PK profiles. The concentration-time curves of the six dosing regimens, simulated by DAS3.0 software, were close to nominal in all instances. The $t_{1/2}$ value was 1.20 ± 0.36 h. For the 0.5-, 1.0-, and 2.0-g dose regimens with a 0.5-h infusion, the C_{max} values for strains CSRA24 and CSRA91 were 24.07 and 24.10, 44.34 and 44.33, and 93.86 and 94.15 $\mu\text{g}/\text{ml}$, respectively. For the 0.5-, 1.0-, and 2.0-g dose regimens with a 3-h infusion, the C_{max} values for strains CSRA24 and CSRA91 were 18.33 and 18.56, 9.91 and 9.87, and 37.13 and 36.10 $\mu\text{g}/\text{ml}$, respectively. The correlation coefficient between the measured and simulated values was 0.9878 ± 0.0147 . Typical concentration-time curves are shown in Fig. 2.

Microbiological response. The MFs for both strains CSRA24 and CSRA91 were approximately 10^{-7} . The effects of the six dosage-time regimens on the total-population burden for strains CSRA24 and CSRA91 are shown in Fig. 3. Initial bacterial counts at baseline were 1×10^9 to 4×10^9 CFU/ml. All dosage-time regimens, except for the lowest dosage (0.5 g), showed 3-log CFU/ml bacterial killing for both strains. For strain CSRA24, the regimens of 1.0 g with a 3-h infusion, 2.0 g with a 0.5-h infusion, and 2.0 g with a 3-h infusion showed a quick 3-log CFU/ml bacterial killing within 24 h, and the regimen of 1.0 g with a 0.5-h infusion attained a 3-log CFU/ml reduction at 72 h. For strain

FIG 3 Effects of various regimens of meropenem on the total-population burden for strains CSRA24 (A) and CSRA91 (B).

CSRA91, all six regimens showed a quick 3-log CFU/ml bacterial killing within 24 h.

Figure 4 illustrates the time courses of killing and enrichment of resistant mutant strains with the drug-free control (Fig. 4A) and six meropenem dosage regimens (Fig. 4B to G). For the two 0.5-g regimens, the resistant mutant strains enriched quickly after 48 h and rose to 10^7 to 10^8 CFU/ml at 168 h (Fig. 4B and C). Although the two 1.0-g regimens displayed 3-log CFU/ml bacterial killing for strains CSRA24 and CSRA91, enrichment of resistant mutant strains developed at 48 h and 72 h, respectively, and later reached 10^6 to 10^7 CFU/ml (Fig. 4D and E). From the baseline to 48 h, the MICs for the bacteria that grew on meropenem-containing plates were 8 μ g/ml for CSRA24 and 4 to 8 μ g/ml for CSRA91. From 72 h to 144 h, the MICs for all bacteria that grew on meropenem-containing plates were ≥ 16 μ g/ml (16 to 64 μ g/ml).

The two 2.0-g regimens, including both the 0.5-h and 3-h infusions, demonstrated obvious bactericidal and resistance-suppressing effects on both strains. The total bacterial count fell to 10^1 to 10^2 CFU/ml for CSRA24 and fell to $<10^1$ CFU/ml (below the detection limit) for CSRA91 at the end of the experiment, and an enrichment of mutant strains was not observed (Fig. 4F and G). The Kaplan-Meier plot, with the Breslow-Gehan test, comparing the cumulated survival rates between regimens of 2.0 g with a 0.5-h infusion and 2.0 g with a 3.0-h infusion during the whole experiment demonstrated that there was no statistical difference between the two regimens ($P = 0.597$ and $P = 1.000$ for strains CSRA24 and CSRA91, respectively) (Fig. 5).

PK/PD indices for resistance suppression. The PK/PD parameters for the six meropenem dosage regimens for both strains are shown in Table 1. The $\%T > \text{MIC}$ of all regimens was $>60\%$ for both strains CSRA24 and CSRA91. For strain CSRA24, the regimen of 2.0 g with a 3-h infusion had a $\%T > \text{MIC}$ of 100%, and the regimens of 1.0 g with a 3-h infusion and 2.0 g with a 0.5-h infusion almost reached a $\%T > \text{MIC}$ of 100%. For strain CSRA91, all 1.0- and 2.0-g regimens had a $\%T > \text{MIC}$ of 100%. However, resistance developed in both strains with the 1.0-g regimens along with the 0.5-g regimens (Table 1). Therefore, we did not consider

$\%T > \text{MIC}$ to be a suitable parameter for resistance suppression. A similar phenomenon was observed for the $C_{\text{min}}/\text{MIC}$ ratio and $\%T_{\text{MSW}}$ (Table 1). The two 2.0-g regimens (both 0.5-h and 3-h infusions) had a $\%T > \text{MPC}$ of $\geq 20\%$ and a $T > \text{MPC}/T_{\text{MSW}}$ ratio of ≥ 0.25 for both strains and displayed rapid bacterial killing and resistance suppression effects, whereas other regimens had a $\%T > \text{MPC}$ of $<20\%$ or a $T > \text{MPC}/T_{\text{MSW}}$ ratio of <0.25 and failed to suppress enrichment of mutant strains, indicating that the $\%T > \text{MPC}$ or the $T > \text{MPC}/T_{\text{MSW}}$ ratio is a suitable PK/PD parameter.

DISCUSSION

In the present study, the two 2.0-g regimens, which achieved a $\%T > \text{MPC}$ of $>20\%$ or a $T > \text{MPC}/T_{\text{MSW}}$ ratio of >0.25 , were observed to exhibit an obvious bactericidal effect and suppressed resistance. MPC is a “farsighted” concept focusing on the prevention of emergence of resistance by modifying antibiotic drug concentrations (25). According to the MPC theory, ideal regimens for antibiotics should involve not only the attainment of clinical efficacy but also the minimization of resistance emergence. However, studies on preventing carbapenem-resistant *A. baumannii* emergence based on MPC are scarce. Credito et al. tested MPCs of four carbapenems against 25 clinical strains of *A. baumannii*. Those researchers found that the MPC_{50} and MPC_{90} of meropenem were 8 and 128 μ g/ml, respectively (26). In the present study, we assessed the PK/PD of meropenem for two clinical CS-MDRAB strains, CSRA24 and CSRA91, and further evaluated six routine clinical regimens for their effects on preventing resistance in the two strains. We observed that these two strains had similar MPCs but different MICs. More importantly, we revealed, for the first time, that the resistance of *A. baumannii* was suppressed by the optimized meropenem dosage regimens of 2 g q8h with a 0.5-h or 3-h infusion and that the $\%T > \text{MPC}$ or the $T > \text{MPC}/T_{\text{MSW}}$ ratio was an optimal PK/PD parameter for suppression of resistance of CS-MDRAB.

In the present study, we applied a modified HFIM to simulate human exposure and test the PK/PD of meropenem. In prelimi-

FIG 4 Time courses of killing and enrichment of mutations with various regimens of meropenem. (A) Drug-free control; (B) 0.5 g with a 0.5-h infusion q8h; (C) 0.5 g with a 3-h infusion q8h; (D) 1.0 g with a 0.5-h infusion q8h; (E) 1.0 g with a 3-h infusion q8h; (F) 2.0 g with a 0.5-h infusion q8h; (G) 2.0 g with a 3-h infusion q8h.

FIG 5 Kaplan-Meier plot showing bacterial killing with meropenem dosage regimens of 2.0 g with a 0.5-h infusion and 2.0 g with a 3-h infusion for strains CSRA24 (A) and CSRA91 (B).

nary experiments, we observed that the degree and speed balance of drugs with a short half-life between the extracapillary and inner capillary compartments were seriously limited. This limitation occurred even when the flow rate of the extracapillary compartment reached the maximum, which could cause discordance in concentration-time curves between the extracapillary and inner capillary compartments. In order to solve this problem, reverse external circulation of the extracapillary compartment with a low flow rate was added. With this modification, the concentration-time curve between the extracapillary and central compartments can be kept consistent. Indeed, in our preliminary experiment, we validated the concordance of the PK profiles of 0.5-h and 3-h infusions of meropenem in the central and extracapillary compartments. The time to reach the $C_{\max}$ in the extracapillary and central compartments was the same. The $t_{1/2}$ values for the extracapillary and central compartments were 1.27 h and 1.25 h, respectively, for the regimen of 2 g with a 0.5-h infusion and 1.22 h and 1.18 h, respectively, for the regimen of 2 g with a 3-h infusion. The $C_{\max}$ values for the extracapillary and central compartments were 85.33 $\mu\text{g/ml}$ and 90.29 $\mu\text{g/ml}$, respectively, for the regimen of 2 g with a 0.5-h

infusion and 37.13 $\mu\text{g/ml}$ and 38.16 $\mu\text{g/ml}$, respectively, for the regimen of 2 g with a 3-h infusion. In addition, we observed that bacteria in the extracapillary compartment tended to concentrate close to the outlet and the bottom of the cartridge when there was only one high-rate flow passing through it. Consequently, the nonhomogeneous bacterial suspension could lead to errors in bacterial sample collection. The addition of reverse external circulation maintains homogeneity in the bacterial suspension in the extracapillary compartment.

The two strains used in the present study had different MICs but the same MPC. Whereas the MIC is the lowest drug concentration inhibiting the growth of 10^5 CFU/ml of the bacterium in *in vitro* measurements, the MPC is the lowest drug concentration preventing the growth of first-step resistant mutants and is measured based on 10^{10} CFU/ml. The factors that influence MPC are not fully understood. It has been reported that MIC correlates poorly with MPC and that MPC cannot be accurately predicted from MIC (27, 28). The two strains used in the present study had different drug resistance spectra and probably contained different susceptible subpopulations that determined the MPCs.

TABLE 1 PK/PD parameters of the six meropenem dosage regimens^b

Strain	Regimen ^a	%T>MIC	$C_{\min}/\text{MIC}$ ratio	%T>MPC	T_{MSW} (%)	$T>\text{MPC}/T_{\text{MSW}}$ ratio	Resistance suppression
CSRA24	0.5 g with 0.5-h infusion	62	0.16	0	62	0	Failure
	0.5 g with 3-h infusion	67	0.27	0	67	0	Failure
	1.0 g with 0.5-h infusion	74	0.47	12	62	0.19	Failure
	1.0 g with 3-h infusion	92	0.87	0	92	0	Failure
	2.0 g with 0.5-h infusion	94	0.78	32	62	0.52	Success
	2.0 g with 3-h infusion	100	1.01	22	78	0.28	Success
CSRA91	0.5 g with 0.5-h infusion	98	0.92	0	98	0	Failure
	0.5 g with 3-h infusion	94	0.72	0	94	0	Failure
	1.0 g with 0.5-h infusion	100	1.3	12	88	0.14	Failure
	1.0 g with 3-h infusion	100	2.914	0	100	0	Failure
	2.0 g with 0.5-h infusion	100	3.06	32	68	0.47	Success
	2.0 g with 3-h infusion	100	3.784	20	80	0.25	Success

^a Meropenem was infused with the designated dose and infusion time at 8-h intervals for 7 days.

^b %T>MIC, percentage of the dosage interval in which drug concentrations are above the MIC; %T>MPC, percentage of the dosage interval in which drug concentrations are above the mutant prevention concentration; $C_{\min}/\text{MIC}$ ratio, ratio of the minimum concentration to the MIC; T_{MSW} , percentage of the dosage interval in which drug concentrations are within the mutant selection window.

The MICs for the strains on plates containing antibiotic at $3\times$ MIC at baseline were 4 $\mu\text{g/ml}$ for CSRA91 and 8 $\mu\text{g/ml}$ for CSRA24. These values are lower than the reported MICs of clinical isolates of meropenem-resistant *A. baumannii* (16 to 128 $\mu\text{g/ml}$) (29, 30) and showed only intermediate- and low-level resistance to meropenem. However, the MICs of the strains on antibiotic-containing plates increased gradually when enrichment occurred and reached 64 $\mu\text{g/ml}$ at the end of the experiments. A similar tendency of variation in susceptibility was observed previously (31), which demonstrates that suboptimal exposure to antibiotics can lead to MIC creep of preexisting populations with reduced susceptibility and facilitate the emergence of resistance.

As a time-dependent antibiotic, a $T>\text{MIC}$ of 40% is used as the best predictor of bacterial killing and the microbiological response of meropenem (32, 33). In our study, the $\%T>\text{MIC}$ of all regimens was $>60\%$. However, the two 0.5-g regimens did not show an obvious bactericidal effect (3-log CFU/ml bacterial killing), which was consistent with findings from two previous studies showing that meropenem monotherapy did not achieve a 3-log CFU/ml bacterial killing effect in *P. aeruginosa* despite $\%T>\text{MIC}$ s of 84% and 100% (16, 17). We believe that the phenomenon was caused by the enrichment of a resistance mutation that developed quickly and exerted an influence on the bactericidal effect.

In general, a prolonged infusion time can optimize $\%T>\text{MIC}$ by attaining a higher $C_{\min}$ for a time-dependent antibiotic with a short $t_{1/2}$. Theoretically, prolongation of the infusion time for the same dosage regimen should improve the $\%T>\text{MIC}$. Compared to the 0.5-h infusion, prolonged infusions can optimize the efficacy of meropenem, especially for the treatment of infection caused by isolated pathogens with an MIC of 16 $\mu\text{g/ml}$ (22, 32). However, when the MIC is 4 $\mu\text{g/ml}$ or less, the regimen of 0.5 g with a 0.5-h infusion can also attain a $T>\text{MIC}$ of 40%. Thus, a lower MIC and a higher dose are associated with a smaller difference in $\%T>\text{MIC}$ s between continuous infusion and intermittent infusion. In the present study, the meropenem MICs for the two clinical strains CSRA24 and CSRA91 were 2.0 and 0.5 $\mu\text{g/ml}$, respectively, which corresponded to similar $\%T>\text{MIC}$ s for the regimens of 2.0 g with a 0.5-h infusion and 2.0 g with a 3-h infusion. We compared the cumulative survival rates for the whole experiment period for these two 2.0-g regimens and found no statistical difference. Due to the fact that the infusion regimen of 2.0 g with a 3-h infusion did not display any advantage in the bacterial killing effect and resistance suppression compared with the infusion regimen of 2.0 g with a 0.5-h infusion, we assume that the regimen of 2.0 g q8h with a 3-h infusion may not be necessary when the MIC value of an isolated clinical strain of *A. baumannii* is 2 $\mu\text{g/ml}$ or less. Therefore, we recommend that a regimen of 2.0 g q8h with a 0.5-h infusion be applied for target antimicrobial therapy when the MIC is known to be ≤ 2 $\mu\text{g/ml}$.

Traditionally, PK/PD parameters based on MIC are used to predict antibiotic efficacy, but there is now increasing interest in trying to use MPC-related PK/PD parameters to minimize the development of resistance. The correlation between MIC and MPC values is poor (6, 27, 34). Theoretically, MPC is more relevant to the development of resistance (24). Indeed, PK/PD parameters based on MPC are more predictive of bacterial resistance than MIC-related PK/PD parameters (8, 35, 36). In the present study, we also found that $\%T>\text{MPC}$ was a more preferred PK/PD parameter for suppressing mutant subpopulation proliferation than $\%T>\text{MIC}$ (Table 1). Previously, the $C_{\min}/\text{MIC}$ ratio of

meropenem was used as a PK/PD parameter to predict the development of *P. aeruginosa* resistance (16). Tam et al. demonstrated that selective amplification of *P. aeruginosa* subpopulations with reduced susceptibility to meropenem was suppressed with a $C_{\min}/\text{MIC}$ ratio of ≥ 6.2 (16). However, Louie et al. reported conflicting results (17): the $C_{\min}/\text{MIC}$ ratio associated with success in an isogenic MexAB pump-overexpressed mutant of *P. aeruginosa* PAO1 wild type was about the same as that associated with failure in the wild-type strains (1.88 versus 2.06, respectively). In the present study, we noted similar $C_{\min}/\text{MIC}$ values between successful and failed regimens (Table 1), suggesting that the $C_{\min}/\text{MIC}$ ratio is not a suitable index for predicting the development of resistance in *A. baumannii* with meropenem treatment.

According to the MPC theory, MSW is a “danger zone” for drug-selective amplification of resistant subpopulations to occur. T_{MSW} is an important parameter in the enrichment of a mutant subpopulation (20, 24). However, we found that compared with the $T>\text{MPC}/T_{\text{MSW}}$ ratio, T_{MSW} was not a suitable parameter relating to the enrichment of a mutant subpopulation, which was in agreement with findings reported previously by Kesteman et al. (37). The inability of T_{MSW} to predict resistant mutant enrichment may be explained by the confounding influence of the actual antimicrobial concentrations at the edges of the selection window (38, 39). MSW is not a range of concentrations associated with a uniform probability of mutation selection; instead, it should be considered a range of concentrations within which multiple factors influence the likelihood of selecting for resistant microbial strains (40).

In the present study, the two 2.0-g q8h meropenem regimens (with 0.5-h or 3-h infusion) displayed resistance suppression for the two CS-MDRAB strains (CSRA24 and CSRA91). The two strains had different MICs but the same MPC, implying that MPC is critical in determining the resistance suppression of meropenem for *A. baumannii*. Louie et al. (17) demonstrated that meropenem monotherapy of 2.0 g q8h (1-h infusion time for PAO1 wild-type strains and 4-h infusion time for MexAB pump-overexpressing mutant strains) could not suppress resistance of *P. aeruginosa*. The MPCs of strains used in their study were unknown, and thus, we could not confirm whether or not a difference in MPC caused the discordant responses to similar meropenem regimens. It is noteworthy that Bulik et al. (41) reported that carbapenemase-producing *Klebsiella pneumoniae* and *P. aeruginosa* had discordant responses to the same meropenem regimen (2.0 g q8h with a 3-h infusion), although they had the same MIC. Those researchers considered that the reduced $\%T>\text{MIC}$ due to hydrolysis of carbapenemase-producing *K. pneumoniae* was the main reason for the discordant responses. However, different resistance mechanisms between *P. aeruginosa* and *K. pneumoniae* may also be responsible for their different responses to the same meropenem regimen, considering that one of the carbapenemase-producing *K. pneumoniae* strains with a $\%T>\text{MIC}$ of $>40\%$ in that study also displayed discordant responses (41). Accordingly, we presume that the different resistance mechanisms between *P. aeruginosa* and *A. baumannii* may also produce different responses to the same meropenem regimen.

In conclusion, administration of 2.0 g meropenem q8h with either a 0.5-h or 3-h infusion has an obvious bactericidal effect and suppresses resistance enrichment of CS-MDRAB. A $T>\text{MPC}$ of $\geq 20\%$ or a $T>\text{MPC}/T_{\text{MSW}}$ ratio of ≥ 0.25 can be used as a parameter for predicting suppression of meropenem resistance in

CS-MDRAB. Our *in vitro* data support the use of a high dosage of meropenem (2.0 g q8h) for the treatment of severe infection caused by CS-MDRAB.

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