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Use of Carbapenems against Clinical, Nontyphoid *Salmonella* Isolates: Results from *In Vitro* and *In Vivo* Animal Studies

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The emergence of multidrug-resistant *Salmonella* isolates has created the need for new therapeutic agents. We evaluated the intracellular activity of four carbapenem compounds against clinical nontyphoid *Salmonella* (NTS) isolates *in vitro* and *ex vivo*. Subsequently, the efficacy of carbapenem treatment against selected *Salmonella* isolates *in vivo* was assessed using a murine peritonitis model. The MIC_{50} and MIC_{90} for doripenem, ertapenem, imipenem, and meropenem against 126 NTS isolates were found to be 0.062 and 0.062, 0.015 and 0.015, 0.5 and 1, and 0.031 and 0.031 μg/ml, respectively. The intracellular killing effect of ertapenem was sustained for 24 h and was superior to that of imipenem, meropenem, and doripenem; its effect was comparable to that of ceftriaxone. Ertapenem demonstrated an excellent pharmacokinetic profile with a percent time above the MIC of 75.5% and an area under the concentration-time curve/MIC ratio of 20,733. When peritoneal exudate cells were examined directly *ex vivo* from mice with *Salmonella*-induced peritonitis, cells from mice treated with ertapenem and ceftriaxone had intracellular and extracellular bacterial counts reduced 10^2- to 10^4-fold and exhibited killing effects similar to each other. The survival rates of mice inoculated with 1×10^5 and 10^6 CFU of a ceftriaxone-susceptible *Salmonella* isolate that were subsequently treated with ertapenem or ceftriaxone were 100% and 90%, respectively. When mice were inoculated with 5×10^4 and 10^5 CFU of a ceftriaxone-resistant and ciprofloxacin-resistant *Salmonella* isolate, mice treated with ertapenem had a higher survival rate than mice treated with ceftriaxone (70% versus 0% and 50% versus 0%, respectively; $P < 0.001$). Our results suggest that ertapenem is at least as effective as ceftriaxone in treating murine *Salmonella* infections and show that further clinical investigations on the potential use of ertapenem in treatment of human *Salmonella* infections are warranted.

Salmonellosis is an important food-borne disease, and its most common clinical manifestation (gastroenteritis) often resolves without antibiotic treatment. However, antimicrobial therapy is indicated for invasive *Salmonella* infections such as bacteremia, vascular infections, and osteomyelitis. In recent years, antimicrobial resistance to fluoroquinolones and extended-spectrum cephalosporins among clinical *Salmonella* isolates has been a serious problem, particularly in Asia (18, 31). According to a case-control cohort study from Denmark, the 2-year mortality rate for people infected with drug-resistant *Salmonella* strains was 4.8 to 10.3 times higher than that for the general population (11). These data suggest that additional antimicrobial agents that are effective against drug-resistant *Salmonella* strains are critically needed.

The majority of antibiotics that demonstrate antimicrobial activity *in vitro* cannot cure human *Salmonella* infections because of their limited intracellular penetration (4). In addition to this limitation, antimicrobial resistance among *Salmonella* isolates is emerging (35). Both of these factors contribute to the increasing clinical challenge of treating antimicrobial-resistant *Salmonella* infections. Novel drugs with good extracellular and intracellular antibacterial activity are urgently needed to treat drug-resistant *Salmonella* infections. According to previous *in vitro* and *in vivo* studies, tigecycline has been shown to achieve high intracellular concentrations and exhibits a promising survival outcome in infected mice compared with traditional ceftriaxone therapy (20, 32). However, the low serum levels of tigecycline reached by currently recommended dosages might pose a clinical concern for treating *Salmonella* bacteremia in humans (23).

Carbapenems have been reported to be active against *Salmonella* species *in vitro* (3). However, clinical data regarding the use of carbapenems in the treatment of invasive *Salmonella* infections are limited. In one published case, imipenem salvage therapy cured an infant with *Salmonella* meningitis who had relapsed after 1 month of cefotaxime treatment (17). Another patient with relapsing spinal osteomyelitis caused by ciprofloxacin-resistant, extended-spectrum-beta-lactamase (ESBL)-producing *Salmonella* was also cured with imipenem treatment after 7 weeks of cefepime therapy (16). Additionally, high cellular-to-extracellular-concentration ratios have also been reported for meropenem (10). Given these results, we believe that the potential use of carbapenems for treating invasive *Salmonella* infections warrants further study. Using ceftriaxone as a comparator, we evaluated the antimicrobial efficacy of ertapenem, imipenem, meropenem, and doripenem against nontyphoid *Salmonella* NTS isolates *in vitro*. Furthermore, we assessed the *in vivo* efficacy of these four carbapenems in the treatment of *Salmonella* peritonitis in mice.

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TABLE 1 Pharmacokinetic data for selected antibiotic dosages in mice and humans

Drug (reference[s])	Published human data			Mice (present study)		
	Dose (g)	C_{max} (μg/ml)[a]	AUC_{0-24} (μg · h/ml)[a]	Dosage	C_{max} (μg/ml)[a]	AUC_{0-24} (μg · h/ml)[a]
Ceftriaxone (5, 36)	1 g	128.7 ± 14.8	971.1 ± 120.6	100 mg/kg q12h	270 ± 50	941.2 ± 138.2
Meropenem (26)	0.5 g	21.1-30	27.2-32	50 mg/kg q6h	26.9 ± 4.7	45.1 ± 9.3
Doripenem (7)	0.5 g	32.6 ± 4.4	71.8 ± 4.4	50 mg/kg q6h	29.1 ± 3.7	66 ± 5.6
Ertapenem (22)	1 g	154.9 ± 22	572.1 ± 68.8	50 mg/kg q6h	146.0 ± 36.4	451.8 ± 52.3

[a] Data are means ± standard deviations.

MATERIALS AND METHODS

Bacterial isolates. Overall, 126 clinical *Salmonella* isolates were collected: 77 were obtained from patients at the Chi Mei Foundation Hospital, and 49 were obtained from patients at the National Cheng Kung University Hospital, Tainan, Taiwan. Isolates were obtained from pus, stool, blood, or other body fluids and included *Salmonella* serogroups A, B, C1, C2, D, E, and G. All isolates were stored at −80°C in Protect bacterial preservers (Technical Service Consultant Limited, Heywood, Lancashire, England). One ceftriaxone- and ciprofloxacin-susceptible bacteremic isolate, *Salmonella enterica* serotype Typhimurium (S129-42), was randomly selected from the 126 isolates and used throughout the study. Another isolate, *S. enterica* serotype Choleraesuis (S1-9210131), obtained from the National Cheng Kung University Hospital, was used for *in vivo* murine experiments. S1-9210131 is resistant to ciprofloxacin (MIC, 32 μg/ml) and ceftriaxone (MIC, 12 μg/ml) (16).

Macrophage cell line. The RAW 264.7 murine macrophage cell line was obtained from the American Type Culture Collection (ATCC). RAW 264.7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS). Medium supplements were purchased from Gibco, Australia.

MICs. An agar dilution method utilizing Mueller-Hinton agar was used to determine the MICs according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (8). Unless otherwise indicated, all antibiotics were purchased from Sigma (St. Louis, MO). The antibiotic concentrations used to determine MICs were as follows: ampicillin, 2 to 256 μg/ml; trimethoprim-sulfamethoxazole, 0.25/1.75 to 16/304 μg/ml; chloramphenicol, 1 to 256 μg/ml; ceftriaxone, 1 to 128 μg/ml; ciprofloxacin, 0.125 to 128 μg/ml (Bayer AG, Frankfurt, Germany); meropenem, 0.015 to 128 μg/ml; imipenem, 0.015 to 128 μg/ml; ertapenem, 0.015 to 128 μg/ml (Merck, Rahway, New Jersey); and doripenem, 0.015 to 128 μg/ml (Shionogi, Osaka, Japan). The antibiotic-containing plates were prepared within 24 h of use and stored at 4°C. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used in each assay as controls.

Intracellular antibiotic antibacterial activity. RAW 264.7 cells were seeded in 24-well plates in DMEM with 10% FBS at a concentration of 1×10^6 cells per well and allowed to adhere for 2 h. The medium was then removed from the wells, and the cells were washed with phosphate-buffered saline (PBS). An inoculum of 1×10^6 CFU of S129-42 was added to each well. After 1 h, the culture plates were washed three times with PBS and incubated in medium containing 100 μg/ml gentamicin for 30 min at 37°C to kill extracellular bacteria. The plates were then washed three additional times with PBS. The MIC of gentamicin for S129-42 was 1 μg/ml. According to previously published data from studies with humans, the maximum serum concentrations (C_{max}) for parenteral administration of 0.4 g ciprofloxacin, 1 g ceftriaxone, 1 g ertapenem, 0.5 to 1 g imipenem, 0.5 g doripenem, and 0.5 to 1 g meropenem are 4.0 (19), 128.7 (6, 36), 154.9 (22), 48 to 60 (2, 13, 27, 37), 32.6 (7), and 26 to 30 μg/ml (14, 26, 37), respectively. Based on these data, the following concentrations were added to the culture plates 4 h later (28): ciprofloxacin, 3 μg/ml; ceftriaxone, 100 μg/ml; ertapenem, 150 μg/ml; imipenem, 50 μg/ml; doripenem, 30 μg/ml; and meropenem, 30 μg/ml. Additionally, two lower concentrations of each drug, 1/2 and 1/10 of the above concentrations, were tested. At selected intervals, the bacterial loads in the wells were assessed. At each time point, cells were washed with ice-cold PBS, and bacteria were released from the cells using a lysis buffer (1% Triton X-100, 20 mM Tris, 0.2 M NaCl, 2 mM EDTA). The lysates containing released bacteria were serially diluted (1:10 in PBS), plated on LB agar plates, and cultured overnight (24). The limit of detection for this plate counting method was 100 CFU/ml.

Pharmacokinetic studies. Because of the poor intracellular killing effect of imipenem and ciprofloxacin, we excluded these two antimicrobial agents from animal studies. For animal studies, female BALB/c mice 6 to 8 weeks of age and weighing between 18 and 20 g were obtained from the Animal Center of the National Science Council, Taipei, Taiwan. In preliminary studies, the 50% lethal dose (LD_{50}) of S129-42 for healthy female BALB/c mice was less than 30 CFU by intraperitoneal infection (data not shown), indicating that female BALB/c mice are susceptible to *Salmonella* infection. To determine the serum concentrations of ceftriaxone, ertapenem, doripenem, and meropenem in these mice, each drug was subcutaneously administered to healthy mice as a single dose as previously described: 100 mg/kg of body weight for ceftriaxone (1) and 50 mg/kg for ertapenem (34), doripenem (12), and meropenem (15). At 0.5, 1, 2, 4, 6, and 8 h postinjection, blood samples from six anesthetized mice per treatment group were collected by cardiac puncture. Antibiotic concentrations were determined using the paper disk diffusion bioassay method; the *E. coli* control strains ATCC 25933 and ATCC 25922 were used as controls (21). All serum samples were assayed in triplicate. The lower limits of detection for the antibiotics were as follows: ceftriaxone, 0.1 μg/ml; ertapenem, 0.05 μg/ml; doripenem, 0.1 μg/ml; and meropenem, 0.02 μg/ml.

Ex vivo analysis of intracellular antibacterial activity in murine peritoneal exudate cells. The intracellular antibacterial activity of each drug was examined in a murine peritonitis model in which healthy mice were infected intraperitoneally with 8.5×10^5 CFU of S129-42. Beginning 6 h later, 50-mg/kg doses of ertapenem, doripenem, or meropenem were administered every 6 h and 100 mg/kg doses of ceftriaxone were administered every 12 h, both subcutaneously, for a total of 72 h. The mice were sacrificed after the last scheduled dosing. The peritoneum of each animal was washed with 5 ml of normal saline, and the extracellular bacterial load in peritoneal fluids was assessed using a plate counting method on LB agar. Peritoneal exudate cells were washed with sterile PBS, and 100 μg/ml of gentamicin was added to kill extracellular bacteria. The cells were washed again with ice-cold PBS, and bacteria were released using a cell lysis buffer. The lysates containing released bacteria were serially diluted (1:10 in PBS), plated on LB agar, and cultured overnight for bacterial counting.

In vivo studies. Two murine experiments were designed to investigate the therapeutic efficacy of ertapenem, doripenem, meropenem, and ceftriaxone for the treatment of *Salmonella* peritonitis. S129-42 and the ceftriaxone- and ciprofloxacin-resistant isolate S1-9210131 were tested at an inoculum of approximately 10^5 CFU. All animal experiments complied with the relevant guidelines of the Republic of China and the Chi Mei Foundation Medical Center Animal Use Policy.

Bacterial suspensions were diluted in fresh Mueller-Hinton broth. A 0.1-ml volume of the bacterial suspension was injected intraperitoneally into individual mice from each of the four antibiotic groups, and each

TABLE 2 Pharmacokinetic profiles of ceftriaxone, ertapenem, meropenem, and doripenem against murine infections with *Salmonella* isolates S129-42 and S1-9210131

Drug and dosage	S129-42				S1-9210131			
	MIC (μg/ml)	C_{max}/MIC	AUC/MIC	$T>$ MIC	MIC (μg/ml)	C_{max}/MIC	AUC/MIC	$T>$ MIC
Ceftriaxone, 100 mg/kg q12h	0.062	4,354	7,070	60.8	12	22.5	36.5	17.4
Meropenem, 50 mg/kg q6h	0.031	868	1,454	50.	0.031	868	1,454	50.1
Doripenem, 50 mg/kg q6h	0.062	469	1,064	36.1	0.25	116	264	25.0
Ertapenem, 50 mg/kg q6h	<0.015	>6,339	>20,733	>75.5	<0.015	>6,339	>20,733	>75.5

group contained 10 mice. Ertapenem, doripenem, and meropenem were subcutaneously injected at a dose of 50 mg/kg every 6 h for 1 week. Ceftriaxone was given every 12 h at a dose of 100 mg/kg every 12 h for 1 week. The number of surviving mice was recorded at 12-h intervals for 2 weeks.

Statistical methods. Data analysis was performed using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL). To compare the effects between different treatment groups, two-way and one-way within-repeated-subjects analysis of variance (ANOVA) tests were applied. The log-rank test was applied to compare the survival rates of different groups. *P* values of less than 0.05 were considered statistically significant.

RESULTS

Bioassays and pharmacodynamic parameters. The values for C_{max} and the area under the curve from 0 to 24 h (AUC_{0-24}) are listed in Table 1 for mice treated with ceftriaxone (100 mg/kg every 12 h), meropenem (50 mg/kg every 6 h), doripenem (50 mg/kg every 6 h), or ertapenem (50 mg/kg every 6 h). Our pharmacokinetic data are comparable to previously published human data. In mice, the time above MIC ($T>$MIC) for ceftriaxone was 60.8% against S129-42 and 17.4% against S1-9210131 (Table 2). At a dose of 50 mg/kg every 6 h, the $T>$MIC of ertapenem for the two *Salmonella* strains was >75.5% and the AUC/MIC ratio was >20,733. Both parameters for ertapenem were higher than those for meropenem or doripenem; these values were far above the recommended pharmacodynamic targets of carbapenem treatment for Gram-negative bacterial infections (25, 29).

Antibacterial activity of carbapenems *in vitro*. The *in vitro* susceptibility data for nine drugs tested against the 126 NTS isolates are listed in Table 3. The susceptibility rates for ceftriaxone and ciprofloxacin were 88.1 and 88.9%, respectively. According to the latest CLSI criteria, all NTS isolates were susceptible to the carbapenem compounds ertapenem, imipenem, meropenem, and doripenem (8).

TABLE 3 MICs against 126 clinical NTS isolates and study isolates S129-42 and S1-9210131[a]

Drug[b]	Nontyphoid *Salmonella* isolates			MIC (μg/ml)	
	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% susceptible[a]	S129-42	S1-9210131
AMP	32	>512	48.8	>256	>256
CHL	8	>256	53.2	256	64
CIP	<0.125	2	88.9	<0.125	>32
CRO	<1	4	88.1	0.062	12
SXT	0.25/4.75	>32/608	69.8	0.5	>16
DOR	0.062	0.062	100	0.062	0.062
EPT	<0.015	<0.015	100	<0.015	<0.015
IMI	0.5	1	100	0.5	0.25
MEM	0.031	0.031	100	0.031	0.031

[a] Susceptibility was determined by the updated breakpoints recommended in the M100-S21 CLSI document (8).
[b] AMP, ampicillin; CRO, ceftriaxone; CIP, ciprofloxacin; CHL, chloramphenicol; SXT, trimethoprim-sulfamethoxazole; EPT, ertapenem; MEM, meropenem; IMI, imipenem; and DOR, doripenem.

Antibacterial activity of intracellular antibiotics. RAW 264.7 cells were incubated with S129-42 at an initial inoculum of 1×10^6 CFU/ml. One hour later, the intracellular bacterial load was determined to be 10^3 to 10^4 CFU/ml. The intracellular bacterial load had increased to 10^4 to 10^5 CFU/ml at 4 h and to 10^6 to 10^7 CFU/ml by 8 h postinoculation (Fig. 1). After 8 h, the RAW 264.7 cells were lysed because of the increasing intracellular bacterial loads; consequently, no intracellular counts were available at the 24-h postinoculation time point. When infected RAW 264.7 cells were incubated with ceftriaxone at concentrations of 100, 50, or 10 μg/ml, the intracellular bacterial counts declined markedly, and the antibacterial effect lasted for 24 h (Fig. 1A). When infected RAW 264.7 cells were incubated with ciprofloxacin at concentrations of 3, 1.5, or 0.3 μg/ml, the intracellular bacterial counts at 24 h postinoculation showed a minimal decrease only at the highest concentration, 3 μg/ml (Fig. 1B). Imipenem and ciprofloxacin were excluded from further animal studies because of their poor intracellular inhibitory effects. Of the four carbapenems tested, only ertapenem exhibited sustained intracellular antibacterial activity at levels similar to those seen with ceftriaxone; this effect was prominent even at the lowest concentration of 15 μg/ml (Fig. 1E).

Intracellular antibacterial activity of murine peritoneal exudate cells *ex vivo*. In the control group (infected mice without antimicrobial therapy), the extracellular and intracellular bacterial counts both increased from 10^6 to 10^7 and 10^4 to 10^5 CFU/ml to 10^7 to 10^8 CFU/ml at 48 h postinoculation. The intracellular colony counts decreased to 10^2 to 10^3 CFU/ml at 72 h postinoculation in the ertapenem and ceftriaxone groups. In contrast, the bacterial counts in the meropenem and doripenem groups increased at 48 and 72 h postinoculation (Fig. 2A). No significant differences in antibacterial activity against intracellular and extracellular *Salmonella* were observed between peritoneal exudate cells isolated at 72 h postinoculation from ertapenem- and ceftriaxone-treated mice ($P = 0.86$, by repeated ANOVA test) (Fig. 2).

Survival rates of mice with *Salmonella*-induced peritonitis. (i) Experiment 1. Animals that received either no therapy or meropenem therapy did not survive beyond 2 days after intraperitoneal inoculation with 1×10^5 CFU of S129-42. Animals treated with doripenem did not survive beyond 8 days postinoculation. However, all mice treated with either ertapenem or ceftriaxone survived at least 14 days postinoculation (Fig. 3A). When the inoculum was increased 10-fold to 1×10^6 CFU, the survival rate was 90% at 14 days postinoculation in the ertapenem- and ceftriaxone-treated groups (Fig. 3B).

The mean survival time of mice in the doripenem-treated group was longer than that of the meropenem-treated group (7.4

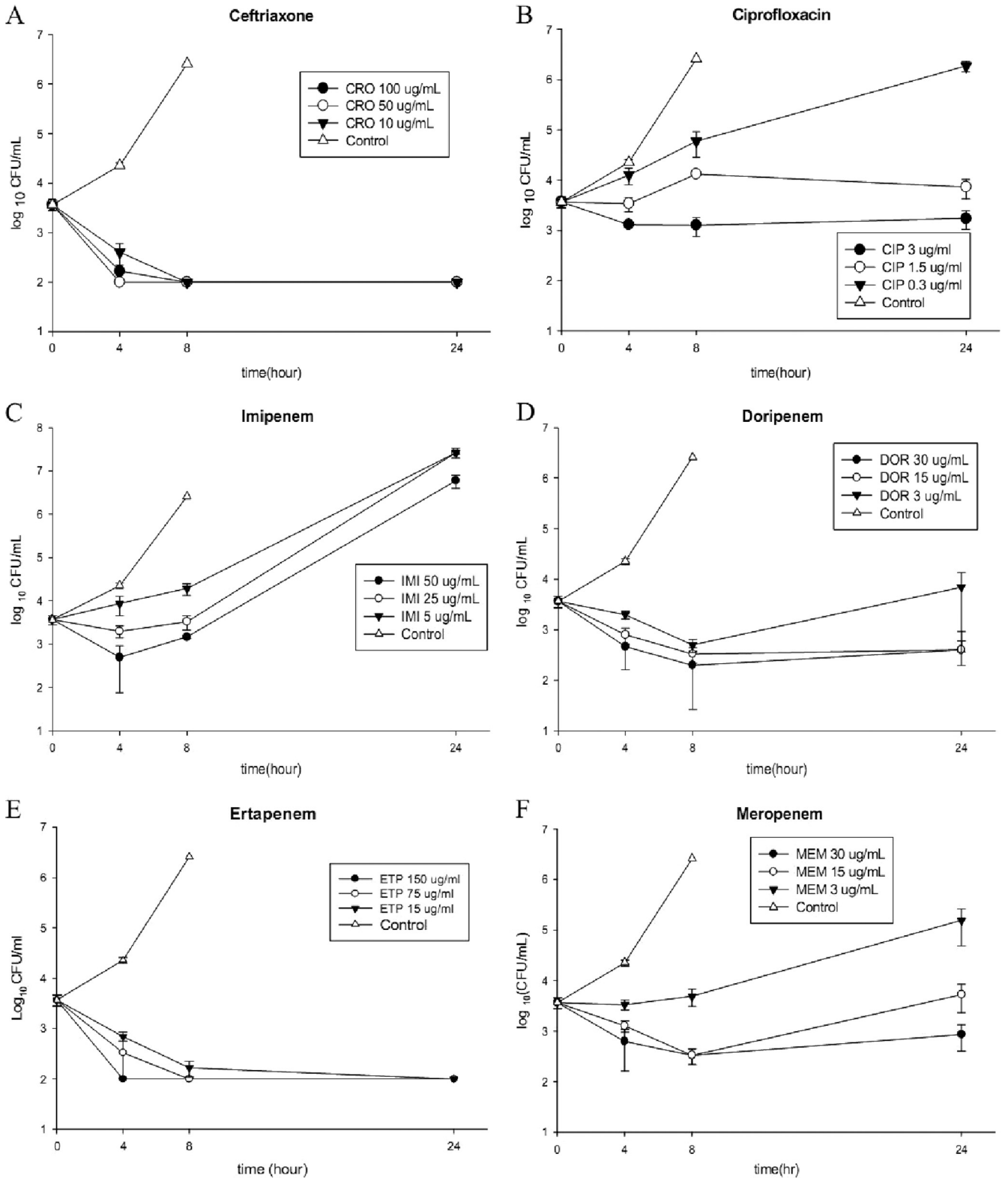

FIG 1 Intracellular antibacterial activities of ceftriaxone (A), ciprofloxacin (B), ertapenem (C), doripenem (D), imipenem (E), and meropenem (F) within RAW 264.7 macrophage cells infected with S129-42 at an inoculum of 1×10^6 CFU/ml. The drug concentrations were as follows: ciprofloxacin, 3 μg/ml; ceftriaxone, 100 μg/ml; ertapenem, 150 μg/ml; imipenem, 50 μg/ml; doripenem, 30 μg/ml; and meropenem, 30 μg/ml. Additionally, 1/2 and 1/10 doses of the original concentrations were tested. All experiments were performed in triplicate. Intracellular bacterial loads are shown as means ± standard deviations.

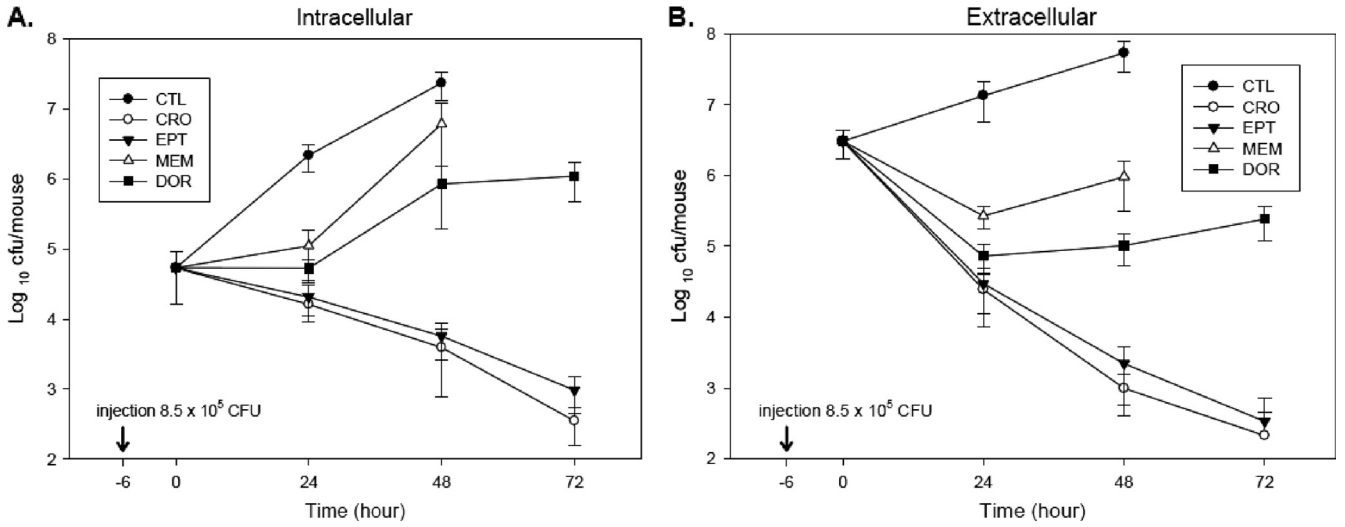

FIG 2 *Ex vivo* intracellular (A) and extracellular (B) antibacterial activity of ceftriaxone, ertapenem, meropenem, and doripenem in a murine peritonitis model. At 6 h after intraperitoneal inoculation of 8.5 × 105 CFU of S129-42, mice were treated subcutaneously with ceftriaxone (100 mg/kg every 12 h), ertapenem (50 mg/kg every 12 h), meropenem (50 mg/kg every 12 h), doripenem (50 mg/kg every 12 h), or saline (control). Intracellular and extracellular counts were evaluated over a period of 72 h.

versus 2 days; $P < 0.001$ by log-rank test). However, the survival rate of mice treated with ertapenem or ceftriaxone at 14 days postinoculation was significantly higher than that of the two aforementioned groups (100% versus 0%; $P < 0.001$ by log-rank test). Therefore, the therapeutic effect of ertapenem was similar to that of ceftriaxone and superior to that of either doripenem or meropenem for the treatment of infection with the ceftriaxone-susceptible *Salmonella* isolate S129-42 (Fig. 3).

(ii) Experiment 2. When mice were infected with an initial inoculum of 5×10^4 CFU of the ceftriaxone- and ciprofloxacin-resistant strain S1-9210131, only 70% of the mice treated with ertapenem survived until day 14 (Fig. 4A). Infecting mice with a 10-fold-concentrated inoculum (5×10^5 CFU) reduced day 14 survival to 50% for the group treated with ertapenem (Fig. 4B). Without therapy or with meropenem, doripenem, or ceftriaxone therapy, no animals survived for more than 10 days irrespective of the inoculum size.

DISCUSSION

Although antimicrobial therapy has been suggested to be indicated only for invasive *Salmonella* infections, not for the more common *Salmonella* gastroenteritis, resistance to expanded-spectrum cephalosporins is emerging among human NTS isolates in Taiwan (5, 35). In a surveillance study, 1.5% of the 3,592 NTS isolates collected between 1999 and 2003 were found to be resistant to ceftriaxone, mainly because of CMY-2 and ESBLs (31). However, ceftriaxone resistance in *S. enterica* serotype Choleraesuis and serogroup B *Salmonella* isolates was shown to have increased by up to 10% in 2009; furthermore, ceftriaxone resistance has also emerged in serotypes other than *S. enterica* serotype Chol-

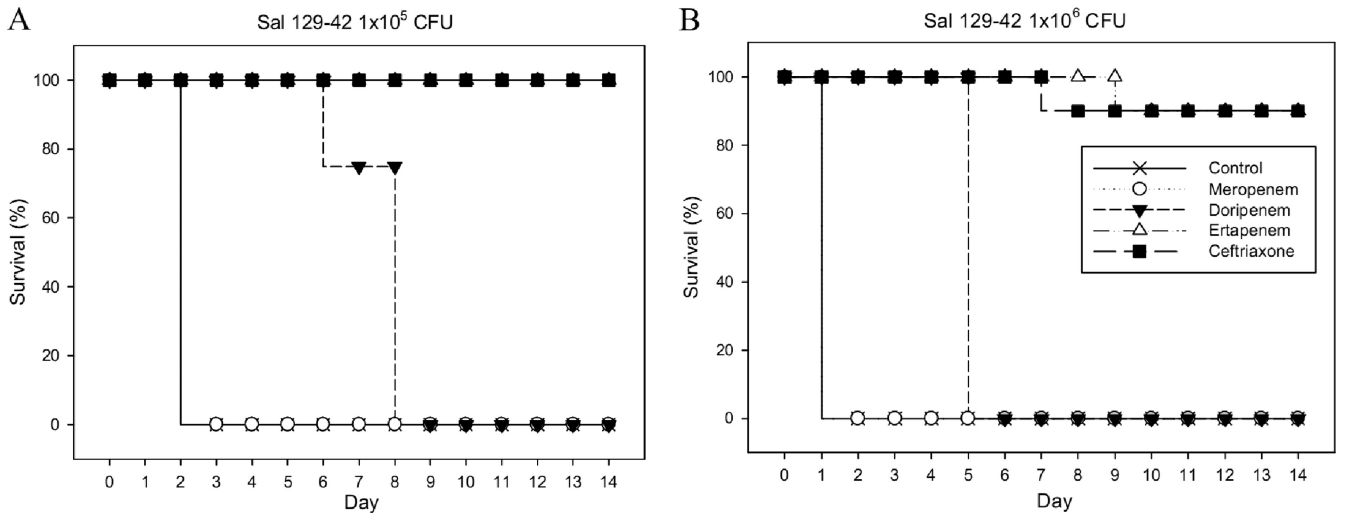

FIG 3 Survival rates of mice (10 per group) that were intraperitoneally infected with 1×10^5 (A) or 1×10^6 (B) CFU of S129-42 and treated with ceftriaxone (100 mg/kg every 12 h), ertapenem (50 mg/kg every 12 h), meropenem (50 mg/kg every 12 h), doripenem (50 mg/kg every 12 h), or saline (control) for 1 week.

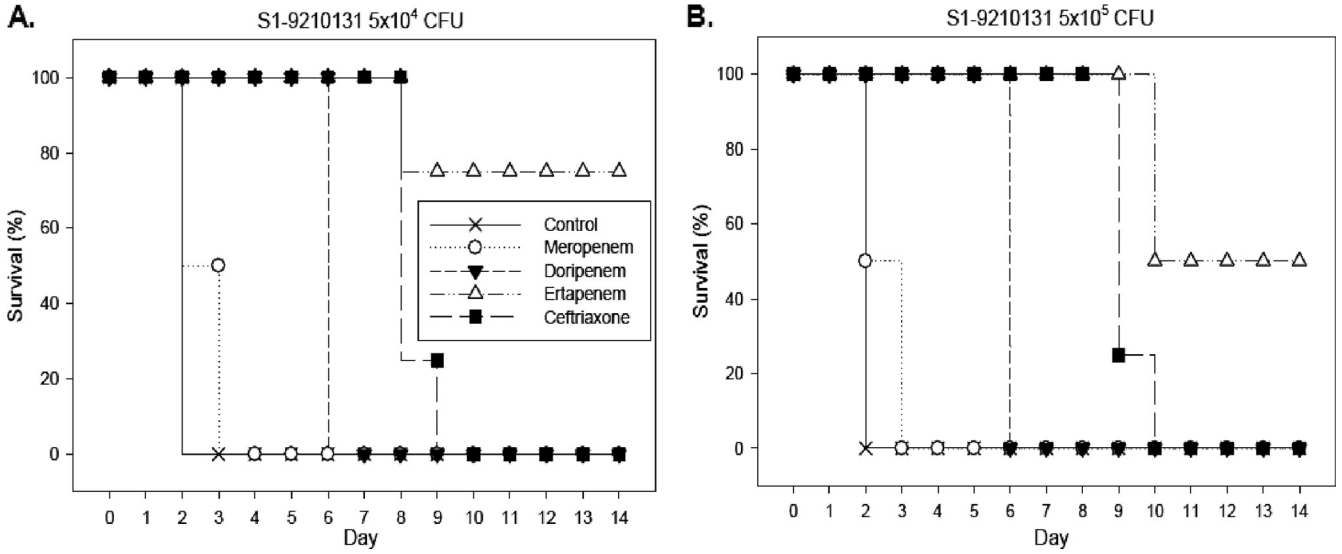

FIG 4 Survival rates of mice (10 per group) intraperitoneally infected with 5×10^4 (A) or 5×10^5 (B) CFU of the ceftriaxone- and ciprofloxacin-resistant strain S1-9210131. Following infection, animals were treated with ceftriaxone (100 mg/kg every 12 h), ertapenem (50 mg/kg every 12 h), meropenem (50 mg/kg every 12 h), doripenem (50 mg/kg every 12 h), or saline (control) for 1 week.

eraesuis (30). These findings are even more concerning in light of the reported concurrent resistance to fluoroquinolones in *S. enterica* serotype Choleraesuis isolates (16). The emergence of antimicrobial resistance to nalidixic acid and ceftriaxone in invasive NTS isolates is also problematic in the United States (9). Collectively, these findings justify the use of carbapenems (the drugs of choice for treating ESBL-producing *Enterobacteriaceae*) in the treatment of human *Salmonella* infections.

In this study, we demonstrated that four carbapenems (ertapenem, imipenem, meropenem, and doripenem) are active against nontyphoid *Salmonella* isolates *in vitro*. The MICs of ertapenem for NTS isolates were lower than those of all other carbapenems tested; consistent with this, the C_{max}/MIC and AUC/MIC values for ertapenem were higher than those for the other three carbapenems. Both *in vitro* in a macrophage cell line and *ex vivo* in murine peritoneal exudate cells, ertapenem showed the best intracellular bactericidal activity among the tested carbapenems, and this activity was similar to that of ceftriaxone. Furthermore, in *ex vivo* experiments, ertapenem achieved these results at levels achievable in serum using the currently recommended dose. In a murine peritonitis model based on infection with a ceftriaxone- and carbapenem-susceptible *Salmonella* isolate, ceftriaxone and ertapenem exhibited therapeutic efficacy superior to that of meropenem or doripenem. Not surprisingly, in mice infected with a ceftriaxone-resistant and carbapenem-susceptible *Salmonella* isolate, ertapenem therapy led to a better outcome than ceftriaxone, meropenem, or doripenem. Our data demonstrate that ertapenem may be a potential alternative for treating human salmonellosis in an era of emerging antimicrobial resistance. However, in light of the species differences between mice and humans, the extrapolation of results from animal studies to clinical situations should be made cautiously. Additional clinical trials involving ertapenem therapy for the treatment of invasive *Salmonella* infections are warranted.

The *in vitro* antibacterial activity of different drugs in macrophage cell lines may be related to the different drug concentration/MIC ratios, though other variables can also affect the intracellular antibacterial activity. In the case of S129-42, the drug concentration/MIC values are low for ciprofloxacin (3/<0.125 = >24) and imipenem (50/0.5 = 100), moderate for doripenem (30/0.062 = 484) and meropenem (30/0.031 = 968), and high for ceftriaxone (100/0.062 = 1,613) and ertapenem (150/<0.015 = >1,000). The latter two drugs exhibited sustained intracellular antibacterial activity for at least 24 h. However, whether using higher doses of imipenem, meropenem, doripenem, or ciprofloxacin will increase their antibacterial activity against intracellular *Salmonella* bacteria requires further study.

Antimicrobial dosages used in a murine model can result in pharmacokinetic profiles similar to previously published data obtained with humans, thus justifying the rationale of drug doses tested in mice. The minimal pharmacokinetic bactericidal target of β-lactam agents for Gram-negative organisms is >40% for the *T*>MIC (29), whereas an AUC/MIC (AUIC) ratio of >125 is associated with preventing the development of antimicrobial resistance (33). In the present study, where we used the pharmacological profile of ceftriaxone as a reference, only ertapenem therapy resulted in a higher *T*>MIC in mice. This finding is consistent with the similarity in therapeutic efficacy of ertapenem and ceftriaxone observed in mice infected with the ceftriaxone-susceptible *Salmonella* isolate. Although meropenem has been shown to exhibit excellent intracellular penetration with a high cellular-to-extracellular-concentration ratio of 3 to 12, in addition to its immunomodulatory activity (10), our results show that its intracellular antibacterial activity against *Salmonella* isolates and its therapeutic efficacy in the treatment of murine peritonitis are inferior to those of ertapenem. However, a higher dose of meropenem, such as 100 mg/kg, may be able to achieve a higher *T*>MIC in mice and improve the survival rate of mice with *Salmonella* peritonitis.

In conclusion, ertapenem is as effective as ceftriaxone in treating mice with *Salmonella* infections, suggesting that it has potential use as a treatment in humans infected with antimicrobial-resistant *Salmonella*.

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We declare no competing interests.

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