In Vitro and *In Vivo* Intracellular Killing Effects of Tigecycline against Clinical Nontyphoid *Salmonella* Isolates Using Ceftriaxone as a Comparator[▽]

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Salmonella is an important, worldwide food-borne pathogen. Resistance to fluoroquinolones and cephalosporins has been increasingly reported, and new therapeutic agents are desperately needed. In this study, we evaluated the *in vitro* antimicrobial susceptibility of clinical nontyphoidal *Salmonella* isolates to tigecycline. Antibacterial activity of tigecycline, ceftriaxone, and ciprofloxacin were investigated by time-kill studies and the murine peritonitis model. The MIC₅₀/MIC₉₀ values of tigecycline, ceftriaxone, and ciprofloxacin against 76 *Salmonella* isolates were 0.25/0.5, 1/8, and 0.125/0.5 µg/ml, respectively. The intracellular inhibitory activity of tigecycline at 0.5 µg/ml (1× MIC) against *Salmonella* isolates in human peripheral blood mononuclear cells was sustained for 24 h. In a mouse peritonitis model, tigecycline reduced the extracellular and intracellular bacterial counts from 10⁷ CFU/ml and 10⁵ CFU/ml, respectively, to an undetectable level within 96 h. The results were similar to those obtained with ceftriaxone. The survival rate of mice exposed to tigecycline after being infected by an inoculum of 1 × 10⁵ CFU was 80%, and that of mice exposed to ceftriaxone was 100%. When the inoculum was increased to 1.3 × 10⁶ CFU, the survival rate of mice treated by tigecycline was 20%, and that of mice exposed to ceftriaxone was 0% (*P* = 0.2). When a ceftriaxone- and ciprofloxacin-resistant but tigecycline-susceptible isolate was tested, mice treated by tigecycline had a higher survival rate than those treated by ceftriaxone (15/20 [75%] versus 6/20 [30%]; *P* = 0.011). Our results suggest that tigecycline is at least as effective as ceftriaxone for murine *Salmonella* infections and warrants further clinical investigations to delineate its potential against human *Salmonella* infections.

Nontyphoidal *Salmonella* species are important food-borne pathogens that cause gastroenteritis, bacteremia, osteomyelitis, or mycotic aneurysm (11, 17, 33). In developed countries, including Taiwan, in recent years there have been significant increases in the resistance rates among antibiotics, including ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol, ceftriaxone, or ciprofloxacin against nontyphoid *Salmonella*, particularly multidrug resistance phenotypes. Among them, ceftriaxone remains to be active against the vast majority of clinical *Salmonella* isolates (2, 6, 14, 27, 30). However, the creeping MIC of ceftriaxone has been noted in Taiwan compared with that in other Asian countries (14). A major cause of this rise in resistance is the widespread use of antimicrobial agents in food animals and in animal feed (27).

Most antibiotics that have been shown to be active *in vitro* cannot cure human *Salmonella* infections due to low intracellular concentrations (4, 9). Tetracycline is usually less susceptible to *Salmonella* than tigecycline, even though it has good intracellular concentration (14). Tigecycline has been reported to be active *in vitro* against *Salmonella* species and has exhibited excellent intracellular concentrations *in vivo* (15, 18, 20).

Therefore, in the present study, using ceftriaxone as a comparator, we examined the *in vitro* and *ex vivo* intracellular killing effect of tigecycline and performed *in vivo* survival studies to evaluate the efficacy of tigecycline in treating nontyphoid *Salmonella* infections in a mouse model.

MATERIALS AND METHODS

Isolates. A total of 77 clinical isolates of *Salmonella* were randomly collected from patients at the Chi-Mei Foundation Hospital, Taiwan. These clinical isolates were originally isolated from blood, stool, pus, or other body fluids and included *Salmonella* serogroups A, B, C1, C2, D, E, and G. All isolates were stored at –70°C in Protect bacterial preservers (Technical Service Consultant Limited, Heywood, Lancashire, England). Two bacteremic isolates, S129-25 and S129-42, were randomly selected. S9210131, an isolate that is resistant to ciprofloxacin (MIC, 32 µg/ml) and ceftriaxone (MIC, 12 µg/ml) (12), obtained from the National Cheng Kung University Hospital, was used for the mouse survival study.

***In vitro* antimicrobial susceptibility.** An agar dilution method utilizing Mueller-Hinton agar was used, according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (7), and phosphate-buffered solution was used to dilute the antibiotics. The concentrations used to determine the MIC values of the tested antibiotics were 2 to 256 µg/ml for ampicillin (Sigma, St. Louis, MO), 1 to 128 µg/ml for ceftriaxone (Sigma, St. Louis, MO), 0.25/1.75 to 16/304 µg/ml for trimethoprim-sulfamethoxazole (Sigma, St. Louis, MO), 0.125 to 128 µg/ml for ciprofloxacin (Bayer AG, Frankfurt, Germany), 1 to 256 µg/ml for chloramphenicol (Sigma, St. Louis, MO), and 0.25 to 64 µg/ml for tigecycline (Wyeth, Puerto Rico). The antibiotic-containing plates were prepared within 24 h and stocked at 4°C before use. *Escherichia coli* ATCC 25922 was used in each assay as a control.

Assessment of the intracellular antibacterial activity of antibiotics. Peripheral blood mononuclear cells (PBMCs) collected from healthy volunteers were iso-

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lated using Ficoll-Plaque (Amersham Pharmacia Biotech, Uppsala, Sweden) and diluted to 1×10^6 cells in 24-well culture plates. PBMCs were incubated with 1×10^7 CFU of S129-42 or S129-25. Thus, the ratio of viable bacteria to PBMCs was 10:1. One hour later, culture plates were incubated with 100 μ g/ml gentamicin for 30 min at 37°C to kill extracellular bacteria and then washed twice with phosphate-buffered saline (PBS). Four hours later, 0.5 μ g/ml tigecycline was added to the culture plates. At selected intervals, bacterial loads in the wells were counted. The cells were washed with ice-cold PBS. Bacteria were released from the PBMCs in lysis buffer (1% Triton X-100, 20 mM Tris, 0.2 M NaCl, 2 mM EDTA), and the lysates with released bacteria were serially diluted (1:10 in PBS), plated on LB agar plates, and cultured overnight prior to bacterial counting (19). The limit of detection via plate counting of bacterial loads was about 10 CFU/ml.

Salmonella in time-kill kinetics. Bacterial suspensions of S129-42 were diluted to around 8×10^4 and 8×10^6 CFU/ml in 25 ml of fresh Mueller-Hinton broth in separate 125-ml glass conical flasks. Susceptible breakpoint concentrations of tigecycline (2 μ g/ml), ceftriaxone (8 μ g/ml), and ciprofloxacin (1 μ g/ml) recommended in the CLSI document M100-S19 were prepared and placed in flasks (7). Each flask was incubated at 37°C. Bacterial counts were measured at 0, 4, 8, 24, and 48 h by enumerating the colony number from 10-fold serially diluted specimens of 100- μ l aliquots plated on Luria-Bertani agar (Difco Laboratories, MI). All experiments were performed at least twice for confirmation of the results.

Pharmacokinetic studies. To determine serum concentrations of tigecycline, ceftriaxone, and ciprofloxacin in mice, each drug was subcutaneously given to healthy mice at a single dose (6.25, 100, and 25 mg of tigecycline, ceftriaxone, and ciprofloxacin/kg body weight, respectively). The dose of tigecycline was selected based on published pharmacokinetic data, which indicated that the dose of 6.25 mg/kg in mice can achieve a maximum concentration of drug in serum ($C_{\max}$) of 1.17 μ g/ml, similar to the $C_{\max}$ of 0.93 μ g/ml at a dose of 100 mg every 12 h in humans (13, 18). For ceftriaxone, the dose of 100 mg/kg in mice can achieve a $C_{\max}$ of 91 μ g/ml, and 0.5 g every 12 h in healthy volunteers can achieve a $C_{\max}$ of 69 to 102 μ g/ml (1, 21, 26). For ciprofloxacin, the dose of 25 mg/kg in mice achieves a $C_{\max}$ of 2.25 μ g/ml, and 750 mg every 12 h in healthy volunteers can achieve a $C_{\max}$ of 2.56 μ g/ml (3, 22). At each time point of 0.5, 1, 2, 4, 6, and 8 h, blood samples from six anesthetized mice were obtained by cardiac puncture. Antibiotic concentrations were determined by the paper-disk diffusion method with two control stains, *E. coli* ATCC 25933 (25922) (for ciprofloxacin and ceftriaxone) and *Bacillus cereus* ATCC 11778 (for tigecycline). All serum samples were assayed in triplicate. The lower limit of detection for ciprofloxacin is 0.15 μ g/ml, that of ceftriaxone is 1.0 μ g/ml, and that of tigecycline is 0.06 μ g/ml.

Intracellular antibacterial activity of tigecycline in a mouse peritonitis model. Female inbred BALB/c mice (Animal Center, National Science Council, Taipei, Taiwan) weighing 18 to 20 g (6 to 8 weeks old) were used throughout the study. In the peritonitis model, we found that the 50% lethal dose (LD_{50}) of S129-42 for healthy female BALB/c mice was less than 30 CFU (data not shown), indicating that female BALB/c mice are susceptible to *Salmonella* infection. Each healthy mouse was infected intraperitoneally with 1.3×10^6 CFU of S129-42. Six hours later, tigecycline (6.25 mg/kg) or ceftriaxone (100 mg/kg) was administered subcutaneously every 12 h for a total of 96 h. The last doses of antibiotics were given at 12 h before each sampling time point. The extracellular bacterial numbers in peritoneal fluids were counted by plating on LB agar. The macrophages in the peritoneal fluid were then washed with sterile PBS, and 100 μ g/ml gentamicin was added to kill extracellular bacteria, after which the cells were washed with ice-cold PBS (19). Bacteria were released from the cells in lysis buffer. The lysates containing released bacteria were serially diluted (1:10 in PBS), plated on LB agar, and cultured overnight prior to bacterial counting.

In vivo mouse peritonitis study. Bacterial suspensions were diluted in fresh Mueller-Hinton broth. A bacterial suspension in a volume of 0.1 ml was delivered intraperitoneally to each healthy mouse. Tigecycline (6.25 mg/kg every 12 h) or ceftriaxone (100 mg/kg every 12 h) was given subcutaneously 6 h after peritoneal infection (21, 23, 32, 33), and 14 doses of each drug were administered. In every group, 7 to 20 mice were tested. The numbers of surviving mice were recorded at 12-hour intervals over 14 days. Two mouse experiments were designed to study the therapeutic efficacy of tigecycline. S129-42, a susceptible isolate, was tested in the following animal studies. In experiment 1, S129-42 was delivered intraperitoneally to 7 to 10 mice at different inocula (1×10^4 , 1×10^5 , and 1.3×10^6 CFU), and the survival rates of infected mice were compared between the control and tigecycline- and ceftriaxone-treated groups. In experiment 2, the survival rates of 20 mice infected by tigecycline-susceptible S9210131 in each of the ceftriaxone-treated, tigecycline-treated, and control groups were studied. These animal experiments complied with all relevant national guidelines of the Republic of China and with the Chi Mei Foundation Medical Center Animal Use Policy.

TABLE 1. MICs for antibiotics against 76 clinical nontyphoid *Salmonella* isolates, including three study isolates

Drug ^a	76 nontyphoid <i>Salmonella</i> isolates			MIC (μ g/ml)		
	MIC ₅₀ (μ g/ml)	MIC ₉₀ (μ g/ml)	% Susceptible ^b	S129-25	S129-42	S9210131
AMP	2	>256	54.5	>256	>256	>256
CHL	4	256	59.7	64	256	64
CIP	<0.125	0.5	93.5	<0.125	0.125	>32
CRO	<1	8	89.6	0.0625	0.0625	12
SXT	<0.25	>16	75.3	>16	0.5	>16
TGC	0.25	0.5		0.5	0.5	0.5

^a Ampicillin (AMP), ceftriaxone (CRO), ciprofloxacin (CIP), chloramphenicol (CHL), trimethoprim-sulfamethoxazole (SXT), and tigecycline (TGC).

^b Susceptible rate is determined by the updated breakpoints recommended in the CLSI document M100-S21 (8).

Statistical methods. Data analyses were performed using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL). To compare the effects of different groups, repeated analysis of variance (ANOVA) tests with two between-subjects and one within-subjects factor (see Fig. 1) and one between-subjects and one within-subjects factor (see Fig. 3) were applied. The log-rank test was applied to compare the survival rates of the different groups. A *P* value of less than 0.05 was considered to be statistically significant.

RESULTS

Bioassay and pharmacodynamic parameters. A single dose of 6.25 mg/kg tigecycline, 100 mg/kg ceftriaxone, or 25 mg/kg ciprofloxacin was given subcutaneously in mice, and the resulting $C_{\max}$ values were 0.9, 90, and 2.5 μ g/ml, respectively. In the mouse model, the time above MIC ($T > \text{MIC}$) for ceftriaxone is 49.5%, close to the minimal pharmacodynamic bactericidal requirement of β -lactam agents for Gram-negative organisms (40%) (10). The 24-h AUC/MIC ratio (area under the concentration-time curve over 24 h in the steady state divided by the MIC), likely the primary determinant of *in vivo* therapeutic efficacy, of tigecycline is 7.23. Such a value was beyond the recommended pharmacodynamic target of tigecycline for Gram-negative bacteria, ≥ 6.96 (10).

In vitro antibacterial and ex vivo intracellular antibacterial activity of tigecycline. The MIC₉₀ values of antibiotics against 76 nontyphoid *Salmonella* isolates were 256 μ g/ml for ampicillin, 256 μ g/ml for chloramphenicol, 8 μ g/ml for ceftriaxone, 16/304 μ g/ml for trimethoprim-sulfamethoxazole, 0.5 μ g/ml for ciprofloxacin, and 0.5 μ g/ml for tigecycline (Table 1). According to the latest CLSI document, M100-S21 (8), the susceptible percentage of ceftriaxone is 89.6%. When S129-42 and S129-25 at an initial inoculum of 1×10^7 CFU/ml were incubated without antibiotic, the intracellular colony counts within PBMCs were 10^4 to 10^5 CFU/ml at 4 h and 10^7 CFU/ml at 24 h. When incubated with tigecycline at a concentration of 0.5 μ g/ml ($1 \times \text{MIC}$), the intracellular bacterial counts decreased from 10^4 CFU/ml at 4 h to 10^2 CFU/ml at 24 h (Fig. 1). The *in vitro* intracellular antibacterial effect of tigecycline against these two isolates was significant ($P < 0.001$) compared with that of the control group, but no differences were observed between two isolates ($P = 0.25$).

Time-kill kinetics. At an initial inoculum of 8×10^6 CFU/ml, the colony count in the tigecycline group decreased to 10^5 CFU/ml at 24 h (Fig. 2). However, the colony count decreased

FIG. 1. Within peripheral blood mononuclear cells, the intracellular bacterial counts of two *Salmonella* isolates, S129-42 and S129-25, with an inoculum of 1×10^7 CFU/ml incubated with 0.5 μ g/ml tigecycline, $1 \times$ MIC for both isolates. All experiments were performed in triplicate, and data are shown as mean values $\pm$ standard deviations.

to an undetectable level at a lower inoculum (8×10^4 CFU/ml) at 24 h. On the other hand, the killing effects of ceftriaxone or ciprofloxacin were more significant, and colony counts were undetectable at 4 h at both inocula, which is suggestive of rapid killing activity.

Ex vivo intracellular antibacterial activity of tigecycline. In the control group, i.e., infected mice without antibiotic therapy, the extracellular/intracellular counts increased from $10^7/10^5$ CFU/ml at 0 h to $10^8/(1.6 \times 10^8/4.3 \times 10^7)$ CFU/ml at 48 h. In the evaluation of the intracellular antibacterial effects of tigecycline and ceftriaxone, we found that colony counts decreased with time to no detectable CFU, compared with that of the control group ($P < 0.001$) (Fig. 3A). On the other hand, there was no difference in antibacterial activity against extracellular *Salmonella* at 72 h between tigecycline- and ceftriaxone-treated mice ($P = 0.92$, by repeated ANOVA test) (Fig. 3B). Tigecycline and ceftriaxone exhibited similar extracellular and intracellular antibacterial effects, as shown in Fig. 3.

Survival rates of mice with *Salmonella* peritonitis. (i) Experiment 1. Without therapy, no mouse survived beyond 7 days after intraperitoneal inoculation of S129-42. The results of our pilot study showed that ceftriaxone was more effective than ciprofloxacin (data not shown); therefore, the killing effect of tigecycline was compared with that of ceftriaxone. At an inoculum of 1×10^4 or 1×10^5 CFU, the survival rate was 100% in the ceftriaxone-treated group and 80% in the tigecycline-treated group. When the inoculum was increased to 1.3×10^6 CFU, the 10-day survival rate in the tigecycline-treated group was 20%, and that in the ceftriaxone-treated group was 0% ($P = 0.2$) (Table 2).

(ii) Experiment 2. Twenty mice were studied in each group, and the initial inoculum was 2×10^5 CFU. Due to the better killing effect of ceftriaxone, tigecycline and ceftriaxone were used to treat mice infected by the antimicrobial-resistant *Salmonella* strain. At day 8, there were no survivors in the untreated group that had received intraperitoneal inoculation of S9210131. After 1 week of therapy, 15 (75%) of the 20 mice in the tigecycline-treated group and 6 (30%) of the 20 mice in the

FIG. 2. Time-kill curves of *Salmonella* S129-42 isolates, with two inocula of 8×10^4 (A) and 8×10^6 (B) CFU/ml, incubated with 2 μ g/ml tigecycline (TGC), 8 μ g/ml ceftriaxone (CRO), or 1 μ g/ml ciprofloxacin (CIP). These drug concentrations were the susceptible breakpoints recommended by the Clinical and Laboratory Standards Institute document, M100-S19. All experiments were performed in duplicate.

ceftriaxone-treated group were alive (75% versus 30%, $P = 0.01$ by log-rank test) (Fig. 4).

DISCUSSION

Many classes of antimicrobial agents, such as ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, ceftriaxone, and ciprofloxacin, have been reported to be active against *Salmonella* species (4). However, recent reports from Taiwan indicate that antimicrobial resistance among clinical *Salmonella* isolates is a serious problem (5, 28, 29, 35). In an era of increasing resistance to fluoroquinolones or extended-spectrum cephalosporins (5, 28, 31, 35), new antibiotics with good extracellular and intracellular antibacterial activity against *Salmonella* isolates are urgently needed. In the literature, tigecycline has been shown to have low MIC values for *Salmonella* and can achieve excellent intracellular concentrations (15, 18, 20). Our MIC data, PBMC study, and the mouse model of

FIG. 3. Intracellular (A) and extracellular (B) antibacterial activity of antibiotics in a mouse *Salmonella* peritonitis model. At 6 h after intraperitoneal inoculation of 1.3×10^6 CFU S129-42, tigecycline (6.25 mg/kg every 12 h), ceftriaxone (100 mg/kg every 12 h), or saline was injected subcutaneously. Intracellular and extracellular counts were evaluated over a period of 96 h. With tigecycline and ceftriaxone therapy, the intracellular and extracellular counts decreased with time. At 96 h, the levels were undetectable.

peritoneal infection support that tigecycline has significant *in vitro* and *in vivo* antibacterial activities against extracellular and intracellular *Salmonella*.

Parenteral ceftriaxone has traditionally been one of recom-

FIG. 4. Survival rates of mice ($n = 20$ in each group) intraperitoneally infected with 2×10^5 CFU of ceftriaxone- and ciprofloxacin-resistant S9210131, followed by treatment with tigecycline (TGC; 6.25 mg/kg every 12 h), ceftriaxone (CRO; 100 mg/kg every 12 h), or normal saline (control) for 1 week. The 14-day survival rates were 75% in the TGC group, 30% in the CRO group, and 0% in the control group ($P = 0.01$, by log-rank test).

mended regimens for treating *Salmonella* infections, because it is one of the cephalosporins with acceptable intracellular penetration (30 to 40%) (16). However, resistance to ceftriaxone is emerging (34). On the other hand, fluoroquinolone-resistant *Salmonella* isolates are prevalent in Taiwan (30). Although the killing effect of ciprofloxacin in the time-kill study revealed that the efficacy of ciprofloxacin was similar to that of ceftriaxone, the antibacterial effects of ceftriaxone in the pilot *in vivo* studies were found to be superior to the effects of ciprofloxacin. Therefore, we chose to compare the effectiveness of tigecycline with that of ceftriaxone. Our data support previous findings that tigecycline has excellent cellular penetration (18, 20) and that the antibacterial activity of tigecycline against *Salmonella* isolates is similar to that of ceftriaxone. When we compared the survival rates of mice infected with ceftriaxone-susceptible *Salmonella* isolates at different inocula followed by treatment with different antibiotics, we found that the therapeutic efficacy of tigecycline was comparable to that of ceftriaxone.

Not surprisingly, in our experimental animal model of peritonitis due to a ceftriaxone-resistant *Salmonella* isolate, we found that tigecycline was more effective than ceftriaxone treatment. In Taiwan, among human nontyphoid *Salmonella*

TABLE 2. Survival rates of mice intraperitoneally infected with 1×10^4 , 1×10^5 , or 1.3×10^6 CFU of S129-42 after tigecycline, ceftriaxone, or normal saline therapy for 1 week

Day	% Survival of mice infected with ^a :								
	1.3×10^6 CFU ($n = 10$ mice)			1.0×10^5 CFU ($n = 10$ mice)			1.0×10^4 CFU ($n = 7$ mice)		
	NS	CRO	TGC	NS	CRO	TGC	NS	CRO	TGC
0	100	100	100	100	100	100	100	100	100
4	0	50	100	0	100	100	100	100	100
7	0	0	80	0	100	100	0	100	100
14	0	0	20	0	80	80	0	100	100

^a TGC, tigecycline (6.25 mg/kg every 12 h); CRO, ceftriaxone (100 mg/kg every 12 h); NS, normal saline.

isolates, fluoroquinolone-resistant isolates are becoming increasingly common (6). Moreover, multiple-drug-resistant species, including those with extended-spectrum cephalosporin resistance, have also been reported in Taiwan (31, 34), thereby decreasing the number of available antibiotics for treatment of extraintestinal infections caused by such *Salmonella* isolates. Tigecycline, a member of a new antibiotic class of glycylcyclines, exhibits good tissue penetration, making it a potential choice for invasive human *Salmonella* infections. Our animal study indicated that the survival rate of mice with ciprofloxacin- and ceftriaxone-resistant *Salmonella enterica* subsp. *enterica* serovar Choleraesuis infection was higher in mice treated with tigecycline than in mice treated with ceftriaxone.

Theoretically, low serum levels of tigecycline with the present recommended dosage may be a problem in treating *Salmonella* bacteremia. Therefore, the potential limitation of tigecycline therapy for human *Salmonella* infections must be considered when treating *Salmonella* bloodstream infections (18, 24, 25). In the view of the obvious differences in pharmacokinetic profiles between mice and humans, the extrapolation of animal studies to clinical situations should be further verified. Clinical trials of tigecycline for treatment of antimicrobial-resistant *Salmonella* infections are warranted.

In conclusion, our *in vitro* and animal studies indicate that tigecycline might be as effective as ceftriaxone against *Salmonella* isolates and could be an option for the treatment of invasive *Salmonella* infections.

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REFERENCES

1. Azoulay-Dupuis, E., et al. 2004. Efficacy of BAL5788, a prodrug of cephalosporin BAL9141, in a mouse model of acute pneumococcal pneumonia. *Antimicrob. Agents Chemother.* **48**:1105–1111.
2. Biedenbach, D. J., M. Toleman, T. R. Walsh, and R. N. Jones. 2006. Analysis of *Salmonella* spp. with resistance to extended-spectrum cephalosporins and fluoroquinolones isolated in North America and Latin America: report from the SENTRY Antimicrobial Surveillance Program (1997–2004). *Diagn. Microbiol. Infect. Dis.* **54**:13–21.
3. Chacon-Moreno, B. E., et al. 2009. Efficacy of ciprofloxacin and moxifloxacin against *Nocardia brasiliensis* in vitro and in an experimental model of actinomycetoma in BALB/c mice. *Antimicrob. Agents Chemother.* **53**:295–297.
4. Chang, H. R., I. R. Vladoianu, and J. C. Pechere. 1990. Effects of ampicillin, ceftriaxone, chloramphenicol, pefloxacin and trimethoprim-sulphamethoxazole on *Salmonella typhi* within human monocyte-derived macrophages. *J. Antimicrob. Chemother.* **26**:689–694.
5. Chiu, C. H., et al. 2002. The emergence in Taiwan of fluoroquinolone resistance in *Salmonella enterica* serotype choleraesuis. *N. Engl. J. Med.* **346**:413–419.
6. Chiu, C. H., T. L. Wu, L. H. Su, J. W. Liu, and C. Chu. 2004. Fluoroquinolone resistance in *Salmonella enterica* serotype Choleraesuis, Taiwan, 2000–2003. *Emerg. Infect. Dis.* **10**:1674–1676.
7. Clinical and Laboratory Standards Institute. 2009. Performance standards for antimicrobial susceptibility testing, 19th informational supplement. CLSI document M100-S19. Clinical and Laboratory Standards Institute, Wayne, PA.
8. Clinical and Laboratory Standards Institute. 2011. Performance standards for antimicrobial susceptibility testing, 21st informational supplement. CLSI document M100-S21. Clinical and Laboratory Standards Institute, Wayne, PA.
9. Easmon, C. S., J. P. Crane, and A. Blowers. 1986. Effect of ciprofloxacin on intracellular organisms: in-vitro and in-vivo studies. *J. Antimicrob. Chemother.* **18**(Suppl. D):43–48.
10. Filho, L. S., J. L. Kuti, and D. P. Nicolau. 2007. Employing pharmacokinetic and pharmacodynamic principles to optimize antimicrobial treatment in the face of emerging resistance. *Braz. J. Microbiol.* **38**:183–193.
11. Hohmann, E. L. 2001. Nontyphoidal salmonellosis. *Clin. Infect. Dis.* **32**:263–269.
12. Ko, W. C., et al. 2005. A new therapeutic challenge for old pathogens: community-acquired invasive infections caused by ceftriaxone- and ciprofloxacin-resistant *Salmonella enterica* serotype choleraesuis. *Clin. Infect. Dis.* **40**:315–318.
13. Koomanachai, P., A. Kim, and D. P. Nicolau. 2009. Pharmacodynamic evaluation of tigecycline against *Acinetobacter baumannii* in a murine pneumonia model. *J. Antimicrob. Chemother.* **63**:982–987.
14. Lee, H. Y., et al. 2009. High rate of reduced susceptibility to ciprofloxacin and ceftriaxone among nontyphoid *Salmonella* clinical isolates in Asia. *Antimicrob. Agents Chemother.* **53**:2696–2699.
15. Liu, C. Y., Y. T. Huang, C. H. Liao, and P. R. Hsueh. 2008. In vitro activities of tigecycline against clinical isolates of *Aeromonas*, *Vibrio*, and *Salmonella* species in Taiwan. *Antimicrob. Agents Chemother.* **52**:2677–2679.
16. Matsumoto, Y., et al. 2001. Mechanism of therapeutic effectiveness of cefixime against typhoid fever. *Antimicrob. Agents Chemother.* **45**:2450–2454.
17. Mead, P. S., et al. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* **5**:607–625.
18. Meagher, A. K., P. G. Ambrose, T. H. Grasela, and E. J. Ellis-Grosse. 2005. Pharmacokinetic/pharmacodynamic profile for tigecycline—a new glycylcycline antimicrobial agent. *Diagn. Microbiol. Infect. Dis.* **52**:165–171.
19. Menashe, O., E. Kaganskaya, T. Baasov, and S. Yaron. 2008. Aminoglycosides affect intracellular *Salmonella enterica* serovars Typhimurium and Virchow. *Antimicrob. Agents Chemother.* **52**:920–926.
20. Ong, C. T., C. P. Babalola, C. H. Nightingale, and D. P. Nicolau. 2005. Penetration, efflux and intracellular activity of tigecycline in human polymorphonuclear neutrophils (PMNs). *J. Antimicrob. Chemother.* **56**:498–501.
21. Patel, I. H., et al. 1981. Pharmacokinetics of ceftriaxone in humans. *Antimicrob. Agents Chemother.* **20**:634–641.
22. Piercy, E. A., R. E. Bawdon, and P. A. Mackowiak. 1989. Penetration of ciprofloxacin into saliva and nasal secretions and effect of the drug on the oropharyngeal flora of ill subjects. *Antimicrob. Agents Chemother.* **33**:1645–1646.
23. Reagan-Shaw, S., M. Nihal, and N. Ahmad. 2008. Dose translation from animal to human studies revisited. *FASEB J.* **22**:659–661.
24. Saliba, R., L. Paasch, and A. El Solh. 2009. Tigecycline attenuates staphylococcal superantigen-induced T-cell proliferation and production of cytokines and chemokines. *Immunopharmacol. Immunotoxicol.* **31**:583–588.
25. Salvatore, C. M., et al. 2009. Tigecycline therapy significantly reduces the concentrations of inflammatory pulmonary cytokines and chemokines in a murine model of *Mycoplasma pneumoniae* pneumonia. *Antimicrob. Agents Chemother.* **53**:1546–1551.
26. Sauve, C., et al. 1996. Efficacies of cefotaxime and ceftriaxone in a mouse model of pneumonia induced by two penicillin- and cephalosporin-resistant strains of *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother.* **40**:2829–2834.
27. Su, L. H., C. H. Chiu, C. Chu, and J. T. Ou. 2004. Antimicrobial resistance in nontyphoid *Salmonella* serotypes: a global challenge. *Clin. Infect. Dis.* **39**:546–551.
28. Su, L. H., et al. 2003. In vivo acquisition of ceftriaxone resistance in *Salmonella enterica* serotype Anatum. *Antimicrob. Agents Chemother.* **47**:563–567.
29. Su, L. H., et al. 2001. Secular trends in incidence and antimicrobial resistance among clinical isolates of *Salmonella* at a university hospital in Taiwan, 1983–1999. *Epidemiol. Infect.* **127**:207–213.
30. Su, L. H., et al. 2005. Increasing ceftriaxone resistance in *Salmonella* isolates from a university hospital in Taiwan. *J. Antimicrob. Chemother.* **55**:846–852.
31. Threlfall, E. J. 2002. Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food- and water-borne infections. *FEMS Microbiol. Rev.* **26**:141–148.
32. van Ogtrop, M. L., et al. 2000. In vivo pharmacodynamic activities of two glycylcyclines (GAR-936 and WAY 152,288) against various Gram-positive and Gram-negative bacteria. *Antimicrob. Agents Chemother.* **44**:943–949.
33. Vugia, D. J., et al. 2004. Invasive *Salmonella* infections in the United States, FoodNet, 1996–1999: incidence, serotype distribution, and outcome. *Clin. Infect. Dis.* **38**(Suppl. 3):S149–S156.
34. Yan, J. J., et al. 2003. Emergence of ceftriaxone-resistant *Salmonella* isolates and rapid spread of plasmid-encoded CMY-2-like cephalosporinase, Taiwan. *Emerg. Infect. Dis.* **9**:323–328.
35. Yang, Y. J., et al. 1998. High rates of antimicrobial resistance among clinical isolates of nontyphoidal *Salmonella* in Taiwan. *Eur. J. Clin. Microbiol. Infect. Dis.* **17**:880–883.