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# Activity of Tigecycline in Combination with Colistin, Meropenem, Rifampin, or Gentamicin against KPC-Producing *Enterobacteriaceae* in a Murine Thigh Infection Model

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**Limited antimicrobials remain active for treating severe infections due to KPC-producing pathogens, and optimal regimens have not been established. In murine thigh infections caused by nine KPC-producing clinical strains of *Enterobacteriaceae* (meropenem MICs, 1 to 4 μg/ml), we evaluated the activities of tigecycline, colistin, meropenem, rifampin, and gentamicin in single and combination regimens lasting for 24 h and 48 h. Rifampin, tigecycline, and gentamicin were the most effective monotherapies, reducing significantly the CFU counts yielded from thighs infected by 88.9 to 100%, 77.8 to 88.9%, and 66.7 to 88.9% of strains, respectively; meropenem and colistin alone exhibited considerably lower performance (significant CFU reduction in 33.3% and 22.2 to 33.3% of the strains, respectively). The addition of rifampin or gentamicin to tigecycline produced synergistic effect in most strains, while antagonism was observed in 33.3 to 44.4% of the strains when colistin was added to tigecycline and in 44.4 to 55.5% of the strains for meropenem combination with tigecycline. Tigecycline combinations with gentamicin or with rifampin caused higher CFU reductions than did tigecycline plus colistin or plus meropenem with almost all strains. Furthermore, tigecycline plus gentamicin was significantly more effective than tigecycline plus colistin or tigecycline plus meropenem in 33.3 to 44.4% and 55.5 to 66.7% of the strains, respectively, while tigecycline plus rifampin significantly outperformed tigecycline plus colistin and tigecycline plus meropenem in 33.3% and 66.7 to 77.8% of the strains, respectively. Overall, our *in vivo* study showed that tigecycline plus rifampin or plus gentamicin is a robust regimen against soft tissue infections caused by KPC-producing strains. The combinations of tigecycline with colistin or meropenem should be considered with caution in clinical practice.**

During the last decade, *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacteriaceae* have become common pathogens in many regions worldwide (1, 2). KPC enzymes confer various levels of resistance to all β-lactams, including carbapenems (2). Moreover, $bla_{KPC}$ genes are often linked with various non-β-lactam resistance determinants, further compromising the antibiotic alternatives that could be used for the treatment of clinically significant infections (2–4). It has been well documented from clinical reports that infections due to KPC producers are commonly associated with therapeutic failures and increased mortality rates (2, 3, 5, 6).

The optimal treatment for infections due to KPC-producing bacteria has not yet been well established (5). Susceptibility data suggest that the treatment of infections caused by KPC producers commonly requires the use of tigecycline, colistin, gentamicin, or meropenem as a last-resort drug (2, 5, 7). Regarding the therapeutic activity of tigecycline combinations against infections caused by *Enterobacteriacae*, data are scarce in the literature, which is derived mainly from *in vitro* studies on carbapenem-susceptible isolates and to a lesser degree from *in vivo* studies and human case reports (8–10). In particular, tigecycline combinations tested *in vitro* produced primarily an indifferent response (8). Nevertheless, *in vitro* synergy occurred when tigecycline was combined with rifampin against *Enterobacter* spp. and with amikacin against *Enterobacter* spp. or *Klebsiella pneumoniae*, while bactericidal synergisms occurred with tigecycline plus colistin against *K. pneumoniae* (11, 12). Data from clinical case reports, although still limited, displayed beneficial activity of tigecycline combined with colistin against *K. pneumoniae* bacteremia (12), while antagonism was extremely rare *in vitro* and was not reported *in vivo* (8). As to *in vitro* data from carbapenemase-producing *Enterobacteriacae*, we have previously reported that a degree of synergism may exist with tigecycline plus colistin but mainly at concentrations 4× MIC (13). Recent data for treatment outcomes of infections due to KPC producers suggested that antibiotic combinations in general proved superior to monotherapies (9, 14). Thus, the existing preliminary evidence suggests that tigecycline combinations with a second antimicrobial may prove useful for the treatment of infections due to KPC producers.

To ascertain the therapeutic value of tigecycline combinations against KPC infections, experimental animal studies would enable better delineation of antimicrobial effects. Previous *in vitro* data indicating that the combinations of tigecycline with either rifampin or an aminoglycoside were favorable led to a suggestion that they should be tested in animal infection models to establish their potential use in clinical situations (8). For this purpose, and based also on the available susceptibility results, we undertook experimental thigh infection studies using tigecycline, colistin, gentami-

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cin, meropenem, and rifampin alone and in tigecycline combinations against KPC-producing *Enterobacteriaceae* clinical strains.

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## MATERIALS AND METHODS

**Bacterial strains and susceptibility testing.** The *in vivo* activities of tigecycline, colistin, gentamicin, meropenem, and rifampin alone and tigecycline combined with colistin, gentamicin, meropenem, and rifampin were tested against nine genetically confirmed KPC-producing clinical strains that included eight *K. pneumoniae* and one *Escherichia coli* strain, using the non-carbapenemase-producing *E. coli* strain ATCC 25922 as a control. The strains were selected from our collection of KPC producers, in order to be epidemiologically unrelated (they were isolated in four distinct hospitals located in north, central, and south Greece) and to exhibit relatively low meropenem MICs (≤4 μg/ml). Susceptibility of the strains to tigecycline, colistin, gentamicin, rifampin, meropenem, imipenem, and ertapenem was determined using the agar dilution method according to CLSI guidelines (15). PCR and sequencing assays showed that all microorganisms produced KPC-2 carbapenemase. The *K. pneumoniae* strains belonged to four different clonal types, as determined previously by pulsed-field gel electrophoresis.

**Murine infection model.** The animal studies were approved by the Greek veterinary authorities and conformed to the Protocol for the Protection and Welfare of Animals. The thigh infection protocol was performed as described previously (16). In brief, 6-week-old, specific-pathogen-free, female Bagg inbred albino c-strain (BALB/c) mice (Harlan, Indianapolis, IN) weighing 23 to 27 g were used in each test group (17). Mice were rendered neutropenic (neutrophil count $< 100/mm^3$) by injecting cyclophosphamide intraperitoneally on day 4 (150 mg/kg of body weight) and on day 1 (100 mg/kg) preinoculation (18, 19). Thigh infections with each strain were produced by injecting 0.1 ml of a bacterial suspension of $10^7$ CFU/ml. The infections were done in triplicate using three mice for each 24-h regimen and another three mice for each 48-h regimen for each of the nine study strains as well as for the ATCC 25922 control. After mice were infected, they were administered subcutaneously tigecycline at 50 mg/kg/24 h (20), gentamicin at 5 mg/kg/12 h (21), colistin methanesulfate at 40 mg/kg/8 h (equivalent to approximately 25 mg/kg/8 h of colistin sulfate; reported doses ranged from 20 mg/kg/8 h of colistin methanesulfate to 40 mg/kg/8 h of colistin sulfate [22, 23]), and meropenem at 200 mg/kg/8 h (reported doses ranged from 100 mg/kg/12 h to 400 mg/kg/8 h [24, 25]) and intraperitoneally rifampin at 25 mg/kg/6 h (22), as described previously, or remained untreated. Mice were humanely euthanized at 24 h and 48 h. Thigh muscles were aseptically excised, homogenized in 10 ml of saline, serially diluted, and cultured quantitatively on antibiotic-free agar plates after serial dilutions, for CFU enumeration. The level of detection for this assay was 100 CFU/thigh.

The thigh CFU titer was expressed as $\log_{10}$ CFU/thigh muscle. A *t* test was used for statistical analysis. For all experiments, an antibiotic scheme (either monotherapy or combination) was considered effective when resulting in a statistically significant reduction of CFU counts ($P < 0.05$) compared with another scheme or without treatment. All statistical analyses were performed using Minitab software (version 13.31). A combination was considered to be synergistic when resulting in a higher reduction of CFU yielded from treated mice than with each of the drugs alone; antagonism was considered when monotherapy caused a higher log CFU reduction than the combination (26). CFU reductions of >3 Δlog were considered to indicate bactericidal levels of activity (10).

## RESULTS

**Susceptibility testing.** The agar dilution MICs of the nine KPC-producing strains used in the study are shown in Table 1. All nine strains had relatively low meropenem MICs (1 to 4 μg/ml) and susceptibility to colistin, gentamicin, and tigecycline, whereas they exhibited relatively high rifampin MICs (≥32 μg/ml).

**TABLE 1** Agar dilution MICs of the study strains to relevant antimicrobials[a]

| Isolate | Agar dilution MIC (μg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | MER | TIG | RIF | GEN | COL | IMP | ERT |
| K1 | 1 | 2 | >32 | 2 | 0.25 | 8 | 8 |
| K2 | 2 | 2 | 32 | 2 | 0.5 | 4 | 8 |
| K3 | 2 | 2 | >32 | 1 | 1 | 2 | 8 |
| K4 | 1 | 1 | 32 | 2 | 2 | 4 | 8 |
| K5 | 2 | 2 | >32 | 1 | 0.5 | 2 | 4 |
| K6 | 4 | 2 | 32 | 4 | 1 | 4 | 16 |
| K7 | 4 | 2 | >32 | 1 | 1 | 2 | 8 |
| K8 | 4 | 2 | >32 | 1 | 2 | 2 | 16 |
| E9 | 2 | 0.5 | 32 | 1 | 0.5 | 2 | 8 |
| ATCC 25922 | 0.06 | 0.12 | 32 | 0.5 | 0.5 | 0.12 | 0.015 |

[a] K, *K. pneumoniae*; E, *E. coli*; TIG, tigecycline; COL, colistin; GEN, gentamicin; MER, meropenem; RIF, rifampin; IMP, imipenem; ERT, ertapenem.

**Activities of single regimens and tigecycline combinations compared with infection in untreated animals.** The performance of antibiotics used as monotherapies and in tigecycline combinations, reflected by a significant reduction of colonies in treated compared with untreated animals, is presented in Table 2. Rifampin monotherapy, although not recommended as a single agent (15), had the best performance at both 24 h (effective [$P < 0.05$] in 8 strains; 88.9%) and 48 h (100%), followed by tigecycline at 24 h (77.8%) and gentamicin at 48 h (88.9%). Colistin and meropenem as monotherapies exhibited considerably lower activity, treating effectively ($P < 0.05$) 33.3% or a lower proportion of mice at 24 h and 48 h. Regarding tigecycline combinations, tigecycline with gentamicin and tigecycline with rifampin were the most effective ones (100% at 24 and 48 h), followed by tigecycline with meropenem (88.9% at 24 h) and tigecycline with colistin (77.8%). The combinations of tigecycline with colistin and tigecycline with meropenem at 48 h exhibited reduced efficacy (66.7%). Also, the combinations of tigecycline with rifampin and tigecycline with gentamicin resulted in higher reductions in bacterial densities grown from infected animals than did the other two combinations used. In particular, the activity of tigecycline with rifampin reached bactericidal levels (>3 Δlog reduction) against all KPC producers, while tigecycline with gentamicin was considered bactericidal in 66.7% of strains at 24 h and 88.9% at 48 h.

**Activity of single regimens compared with other monotherapies.** The direct comparison of antibiotic monotherapies with each other after 24 h and 48 h of treatment is presented in Table 3. Tigecycline was significantly more effective ($P < 0.05$) at 24 h and 48 h than were colistin (in 77.8% and 66.7% of strains at each time point, respectively), meropenem (66.7% and 77.8%, respectively), and gentamicin (66.7% at 24 h and 48 h). Tigecycline was similarly effective with rifampin at 24 h (more effective than rifampin [$P < 0.05$] in 33.3% of strains and less effective also in 33.3% of strains) and more effective than rifampin at 48 h (more effective than rifampin in 33.3% of strains, while rifampin was more effective in 11.1% of strains). Rifampin was more effective than colistin (88.9% of strains), gentamicin (66.7%), and meropenem (88.9%) at 24 h, but at 48 h rifampin lost a part of its activity; still, it remained more effective than colistin (44.4% of strains), meropenem (66.7%), and gentamicin (33.3%).

**TABLE 2** Efficacies of antibiotic monotherapies and of tigecycline combinations after 24 and 48 h of treatment vs results for untreated animals[a]

| | TIG vs no treatment | | COL vs no treatment | | GEN vs no treatment | | MER vs no treatment | | RIF vs no treatment | | TIG + COL vs no treatment | | TIG + GEN vs no treatment | | TIG + MER vs no treatment | | TIG + RIF vs no treatment | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolate | Δlog | *P* value[b] | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value |
| 24 h of treatment | | | | | | | | | | | | | | | | | | |
| K 1/4089 | 0.16 | 0.644 | −0.001 | 0.990 | −0.05 | 0.590 | 0.02 | 0.558 | −2.41 | **0.002** | −0.16 | 0.071 | −1.87 | **0.003** | −1.36 | **0.004** | −4.81 | **<0.001** |
| K 2/4179 | −0.24 | 0.137 | −0.11 | 0.068 | −0.39 | 0.256 | −0.24 | 0.137 | −2.25 | **<0.001** | −3.14 | **0.023** | −4.42 | **0.007** | −1.81 | 0.077 | −3.87 | **0.007** |
| K 3/3268 | −2.51 | **0.032** | 0.85 | **0.002** | −1.51 | **0.002** | −0.15 | 0.182 | −2.55 | **0.002** | −2.75 | 0.109 | −4.72 | **0.004** | −1.38 | **0.004** | −4.54 | **<0.001** |
| K4/3712 | −5.23 | **<0.001** | 0.41 | 0.414 | −1.38 | **0.046** | −0.60 | 0.080 | −2.66 | **0.002** | −3.45 | **0.001** | −4.20 | **<0.001** | −2.03 | **0.002** | −5.84 | **<0.001** |
| K5/2458 | −2.98 | **0.003** | −0.52 | 0.052 | −1.02 | **<0.001** | −1.33 | **0.003** | −3.03 | **0.001** | −3.80 | **0.003** | −4.86 | **<0.001** | −3.56 | **0.001** | −5.17 | **<0.001** |
| K6/3189 | −5.48 | **<0.001** | −1.11 | **0.015** | −1.36 | **0.019** | −1.12 | **0.003** | −3.48 | **<0.001** | −4.74 | **0.001** | −5.86 | **<0.001** | −4.87 | **<0.001** | −5.71 | **<0.001** |
| K7/2868 | −2.62 | **0.006** | −1.47 | **0.023** | −0.88 | 0.112 | −0.27 | 0.341 | −0.98 | 0.143 | −2.38 | **0.025** | −3.09 | **<0.001** | −2.68 | **<0.001** | −3.45 | **0.008** |
| K8/3342 | −1.55 | **0.006** | −0.44 | 0.184 | −1.96 | **0.004** | −0.84 | 0.112 | −1.55 | **0.011** | −1.04 | **0.001** | −1.90 | **0.027** | −1.27 | **0.038** | −4.20 | **<0.001** |
| E1/1098 | −2.60 | **0.001** | 0.12 | 0.588 | −1.52 | **0.029** | −1.77 | **0.004** | −2.44 | **0.004** | −3.11 | **0.015** | −2.52 | **0.005** | −2.67 | **0.029** | −3.10 | **<0.001** |
| ATCC 25922 | −2.37 | **0.003** | −0.62 | 0.394 | −0.77 | 0.305 | −4.15 | **0.001** | −1.63 | **0.004** | −4.05 | **0.015** | −5.04 | **<0.001** | −6.05 | **<0.001** | −4.81 | **<0.001** |
| 48 h of treatment | | | | | | | | | | | | | | | | | | |
| K 1/4089 | 0.26 | −0.844 | −0.10 | 0.224 | −0.24 | 0.558 | −0.67 | 0.053 | −2.10 | **0.035** | −0.18 | 0.275 | −4.28 | **0.003** | −0.11 | 0.105 | −4.47 | **<0.001** |
| K 2/4179 | 0.101 | 0.233 | −0.79 | 0.262 | −0.87 | **0.003** | −0.15 | 0.195 | −1.60 | **0.027** | −3.13 | **0.018** | −4.58 | **0.014** | −0.98 | 0.08 | −5.60 | **<0.001** |
| K 3/3268 | −3.70 | **<0.001** | −0.76 | 0.182 | −1.15 | **0.001** | −0.58 | 0.337 | −1.75 | **0.014** | −1.90 | 0.169 | −3.58 | **0.022** | −3.38 | **<0.001** | −3.76 | **0.001** |
| K4/3712 | −5.54 | **<0.001** | −5.22 | **<0.001** | −3.02 | **0.001** | 0.01 | 0.913 | −2.27 | **0.014** | −6.10 | **<0.001** | −7.08 | **<0.001** | −5.91 | **0.001** | −7.11 | **<0.001** |
| K5/2458 | −5.67 | **0.002** | −1.67 | **0.026** | −1.90 | **0.004** | −1.24 | **<0.001** | −3.90 | **0.002** | −5.89 | **0.001** | −6.77 | **<0.001** | −5.70 | **<0.001** | −6.19 | **<0.001** |
| K6/3189 | −3.94 | **<0.001** | −0.21 | 0.495 | −2.90 | **0.028** | −1.37 | **0.011** | −3.76 | **0.002** | −4.09 | **0.015** | −5.65 | **<0.001** | −2.70 | **<0.001** | −6.49 | **<0.001** |
| K7/2868 | −2.96 | **0.010** | −2.18 | **0.014** | −2.72 | **0.036** | −0.23 | 0.161 | −2.78 | **0.003** | −2.31 | **0.015** | −4.62 | **<0.001** | −1.44 | **0.025** | −4.66 | **<0.001** |
| K8/3342 | −0.24 | 0.054 | −0.91 | 0.266 | −2.77 | **<0.001** | −0.52 | 0.217 | −3.22 | **0.002** | −1.22 | **0.015** | −2.51 | **0.003** | −1.54 | 0.058 | −3,98 | **0.002** |
| E1/1098 | −4.57 | **0.008** | 0.27 | 0.399 | −1.86 | **0.027** | −2.12 | **0.007** | −4.55 | **<0.001** | −0.68 | 0.457 | −4.10 | **0.010** | −3.95 | **0.001** | −5.16 | **<0.001** |
| ATCC 25922 | −3.95 | **0.002** | −0.99 | **0.029** | −1.40 | 0.121 | −3.85 | **<0.001** | −2.80 | **0.024** | −5.56 | **0.002** | −6.27 | **<0.001** | −6.23 | **<0.001** | −6.00 | **<0.001** |

[a] K, *K. pneumoniae*; E, *E. coli*; TIG, tigecycline; COL, colistin; GEN, gentamicin; MER, meropenem; RIF, rifampin.
[b] A *P* value of <0.05 indicates significance and is shown in bold.

**Activities of tigecycline combinations compared with monotherapies.** The comparison of the efficacies of tigecycline combinations in comparison with monotherapies after 24 and 48 h of treatment is presented in Table 4. In brief, the addition of colistin to tigecycline did not improve essentially tigecycline performance, while colistin antagonized tigecycline in as many as 44.4% of strains at 48 h. The addition of gentamicin to tigecycline improved tigecycline in 33.3% (24 h) to 44.4% (48 h) of strains. Further, tigecycline plus rifampin was more effective ($P < 0.05$) than tigecycline alone in 44.4% (24 h) to 66.7% (48 h) of strains. The addition of meropenem to tigecycline did not alter considerably the activity of tigecycline, while it antagonized tigecycline in 22.2% of strains at both 24 h and 48 h. Colistin, which was inactive as monotherapy, performed particularly well when combined with tigecycline (88.9% efficiency). Of interest, gentamicin and rifampin, which were effective as monotherapies, when combined with tigecycline exhibited a significantly improved performance after both 24 h and 48 h.

**Comparison of tigecycline combinations.** In Table 5, we compare the efficacies of tigecycline combinations after 24 and 48 h of treatment. In brief, the combination of tigecycline with gentamicin was superior to tigecycline with colistin against infections by 88.9% of strains and significantly more effective ($P < 0.05$) in 33.3% of strains after 24 h, while at 48 h it was superior in all strains and more effective in 44.4% of strains. The combination of tigecycline with gentamicin was superior to tigecycline with mero-

**TABLE 3** Comparison of the efficacies of antibiotic monotherapies after 24 and 48 h of treatment[a]

| | TIG vs COL | | TIG vs GEN | | TIG vs MER | | TIG vs RIF | | COL vs GEN | | COL vs MER | | COL vs RIF | | GEN vs MER | | GEN vs RIF | | MER vs RIF | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolate | Δlog | *P* value[b] | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value |
| 24 h of treatment | | | | | | | | | | | | | | | | | | | | |
| K 1/4089 | 0.14 | 0.723 | 0.19 | 0.070 | 0.11 | 0.746 | 1.55 | **0.023** | 0.05 | 0.451 | −0.02 | 0.638 | 2.41 | **0.001** | −0.07 | 0.426 | 2.36 | **0.001** | 2.43 | **0.001** |
| K 2/4179 | −0.10 | 0.262 | 0.24 | 0.627 | 0.04 | 0.423 | 1.06 | **0.005** | 0.28 | 0.379 | 0.07 | 0.246 | 2.13 | **<0.001** | −0.20 | 0.546 | 1.86 | **0.009** | 2.06 | **0.001** |
| K 3/3268 | −3.31 | **0.025** | −2.12 | **0.048** | −2.31 | **0.049** | −0.27 | 0.126 | 2.36 | **<0.001** | 1.00 | **<0.001** | 3.40 | **0.001** | −1.36 | **0.002** | 1.04 | 0.066 | 2.40 | **0.002** |
| K4/3712 | −4.56 | **<0.001** | −3.57 | **0.010** | −3.92 | **0.001** | −2.16 | **0.002** | 1.79 | **0.010** | 1.04 | **0.034** | 3.07 | **0.004** | −0.79 | 0.070 | 1.28 | 0.090 | 2.07 | **0.007** |
| K5/2458 | −2.46 | **0.001** | −1.95 | **0.008** | −1.65 | **0.003** | 0.05 | 0.90 | 0.50 | 0.055 | 0.80 | **0.006** | 2.51 | **0.002** | 0.30 | 0.140 | 2.00 | **0.001** | 1.70 | **0.010** |
| K6/3189 | −4.37 | **<0.001** | −4.12 | **0.001** | −4.36 | **0.001** | −1.99 | **0.001** | 0.25 | 0.071 | 0.01 | 0.977 | 2.37 | **0.001** | −0.24 | 0.580 | 2.12 | **0.004** | 2.36 | **0.002** |
| K7/2868 | −2.25 | **0.017** | −0.51 | 0.487 | −1.58 | **0.017** | −1.85 | **0.002** | 1.94 | **0.021** | 1.17 | 0.072 | 2.44 | **0.022** | −0.82 | 0.247 | 2.19 | **0.008** | 0.47 | 0.362 |
| K8/3342 | −1.70 | **0.022** | −1.22 | **0.021** | −1.55 | **0.023** | 1.15 | **0.004** | −0.08 | 0.658 | 0.85 | 0.096 | 2.88 | **0.006** | −0.58 | 0.461 | 1.44 | **0.017** | 2.14 | **0.042** |
| E1/1098 | −3.10 | **0.005** | −2.87 | **0.001** | −1.90 | 0.052 | −0.15 | 0.430 | 0.29 | 0.362 | 1.94 | **0.001** | 0.84 | 0.094 | −2.11 | **0.036** | 0.63 | 0.092 | 1.93 | **0.009** |
| ATCC 25922 | −1.75 | 0.114 | −1.61 | 0.145 | 1.77 | **0.007** | −0.74 | 0.079 | 0.14 | 0.878 | 3.52 | **0.024** | 1.01 | 0.170 | 3.38 | **0.014** | 0.87 | 0.278 | −2.51 | **0.003** |
| 48 h of treatment | | | | | | | | | | | | | | | | | | | | |
| K 1/4089 | −1.26 | 0.177 | −1.12 | 0.346 | −0.69 | 0.422 | 0.387 | 0.707 | 0.14 | 0.727 | 0.56 | 0.60 | 2.00 | **0.033** | 0.42 | 0.467 | 1.86 | **0.002** | 1.44 | 0.120 |
| K 2/4179 | 0.90 | 0.187 | 0.98 | **0.001** | 0.26 | 0.094 | 1.70 | **0.019** | 0.08 | 0.892 | −0.64 | 0.371 | 0.80 | 0.316 | −0.72 | **0.030** | 0.73 | 0.181 | 1.45 | **0.030** |
| K 3/3268 | −2.69 | **0.032** | −2.50 | **0.002** | −2.82 | **0.039** | −2.10 | **0.020** | 0.39 | 0.436 | −0.18 | 0.091 | 0.99 | 0.276 | −0.57 | 0.345 | 0.60 | 0.197 | 1.17 | 0.247 |
| K4/3712 | 0.55 | 0.211 | −1.76 | **0.042** | −4.65 | **0.003** | −2.44 | **0.049** | −2.19 | **0.004** | −5.23 | **<0.001** | −2.95 | **0.006** | −3.04 | **0.003** | −0.76 | 0.236 | 2.28 | **0.014** |
| K5/2458 | −4.01 | **0.015** | −3.76 | **0.008** | −4.43 | **0.004** | −1.77 | 0.132 | 0.24 | 0.721 | −0.42 | 0.369 | 2.24 | **0.001** | −0.66 | 0.073 | 1.99 | **0.046** | 2.66 | **0.007** |
| K6/3189 | −3.73 | **0.001** | −1.05 | 0.255 | −2.57 | **0.002** | −0.18 | 0.613 | 2.68 | **0.009** | 1.16 | **<0.001** | 3.55 | **0.007** | −1.52 | **0.050** | 0.87 | 0.426 | 2.39 | **0.018** |
| K7/2868 | −2.75 | **0.015** | −2.38 | **0.020** | −2.10 | **0.044** | −1.92 | **0.029** | −2.36 | **0.031** | −1.09 | 0.184 | 0.48 | 0.468 | −0.66 | 0.148 | 2.17 | **0.012** | 0.26 | 0.571 |
| K8/3342 | −0.30 | **0.018** | −0.36 | **0.025** | −0.97 | **0.024** | −1.02 | 0.317 | −0.15 | 0.562 | 0.73 | 0.211 | 1.66 | **0.019** | −0.74 | 0.166 | 0.28 | 0.475 | 1.12 | **0.036** |
| E1/1098 | −4.16 | **0.001** | −4.01 | **0.003** | −3.23 | **0.003** | −0.35 | 0.481 | 0.54 | 0.361 | 1.09 | **0.027** | −0.74 | 0.232 | −2.55 | **0.019** | 0.72 | 0.226 | 1.37 | **0.041** |
| ATCC 25922 | −2.95 | **0.009** | −2.55 | **0.036** | −0.09 | 0.787 | −1.14 | 0.249 | 0.41 | 0.421 | 2.86 | **0.001** | 1.82 | 0.111 | 2.45 | **0.021** | 1.41 | 0.275 | −1.04 | 0.190 |

[a] K, *K. pneumoniae*; E, *E. coli*; TIG, tigecycline; COL, colistin; GEN, gentamicin; MER, meropenem; RIF, rifampin.
[b] A *P* value of <0.05 indicates significance and is shown in bold.

**TABLE 4** Comparison of the efficacies of tigecycline combinations vs monotherapies after 24 and 48 h of treatment[a]

| Isolate | TIG vs TIG + COL | | TIG vs TIG + GEN | | TIG vs TIG + MER | | TIG vs TIG + RIF | | COL vs TIG+COL | | GEN vs TIG + GEN | | MER vs TIG + MER | | RIF vs TIG + RIF | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Δlog | *P* value[b] | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value |
| 24 h of treatment | | | | | | | | | | | | | | | | |
| K 1/4089 | 0.27 | 0.534 | 1.98 | **0.048** | 1.48 | **0.038** | 2.83 | **0.002** | 0.63 | **0.036** | 1.79 | **0.004** | 1.35 | **<0.001** | 1.28 | **0.006** |
| K 2/4179 | 2.97 | **0.026** | 4.16 | **0.010** | 1.57 | 0.094 | 1.86 | **0.044** | 2.80 | **0.008** | 4.22 | **0.006** | 1.57 | 0.094 | 0.62 | 0.315 |
| K 3/3268 | 1.00 | 0.081 | 2.21 | 0.095 | −1.13[c] | 0.148 | 1.72 | **<0.001** | 3.87 | **0.013** | 3.21 | **0.001** | 1.14 | **0.001** | 1.98 | **0.001** |
| K4/3712 | 0.05 | 0.918 | 0.80 | 0.074 | −1.37[c] | **0.002** | 0.62 | 0.125 | 5.46 | **0.001** | −0.93[c] | 0.054 | 0.47 | 0.135 | 2.77 | **0.002** |
| K5/2458 | 0.83 | 0.307 | 1.88 | **0.027** | 0.58 | 0.364 | 2.19 | **0.011** | 3.28 | **0.008** | 3.83 | **0.001** | 2.23 | **0.009** | 2.14 | **0.001** |
| K6/3189 | −0.74[c] | 0.108 | 0.38 | 0.136 | −0.61[c] | **0.014** | 0.22 | 0.065 | 3.63 | **0.001** | 4.50 | **0.001** | 3.75 | **0.001** | 2.22 | **<0.001** |
| K7/2868 | −0.24[c] | 0.515 | 0.46 | 0.199 | 0.07 | 0.843 | 0.48 | 0.182 | 2.01 | 0.063 | 0.98 | 0.100 | 1.65 | **<0.001** | 0.44 | 0.502 |
| K8/3342 | −0.51[c] | 0.186 | 0.35 | 0.614 | −0.28[c] | 0.597 | 2.38 | **0.008** | 1.69 | **0.003** | 1.57 | 0.061 | 1.27 | **0.024** | 2.02 | **0.002** |
| E1/1098 | 0.52 | 0.090 | 0.17 | 0.559 | 0.56 | 0.390 | 0.18 | 0.398 | 3.25 | **0.005** | 2.80 | **<0.001** | 2.31 | **0.035** | 2.96 | **<0.001** |
| ATCC 25922 | 1.68 | 0.155 | 2.67 | **0.006** | 3.68 | **0.010** | 2.43 | **0.005** | 3.43 | **0.027** | 4.28 | **0.006** | 1.90 | **0.037** | 3.17 | **<0.001** |
| 48 h of treatment | | | | | | | | | | | | | | | | |
| K 1/4089 | −0.51[c] | 0.561 | 2.17 | 0.134 | −1.58[c] | 0.230 | 2.55 | **0.008** | 1.02 | 0.322 | 6.65 | **0.001** | −0.60[c] | 0.062 | 2.17 | **0.033** |
| K2/4179 | 3.07 | **0.004** | 4.88 | **0.001** | 1.15 | **0.004** | 5.46 | **<0.001** | 2.16 | **0.040** | 3.90 | **0.001** | 0.90 | **0.031** | 3.76 | **0.001** |
| K 3/3268 | −0.90[c] | 0.429 | −1.21[c] | 0.848 | −0.18[c] | 0.349 | 0.80 | **0.016** | 1.60 | 0.282 | 1.84 | 0.074 | 9.91 | **0.015** | 2.89 | **0.008** |
| K4/3712 | 0.97 | 0.110 | 1.28 | 0.153 | 0.24 | 0.611 | 1.56 | **0.001** | 0.42 | 0.053 | 3.16 | **0.001** | 5.04 | **0.002** | 3.95 | **0.003** |
| K5/2458 | 0.22 | 0.787 | 1.10 | 0.155 | 0.03 | 0.964 | 0.52 | 0.435 | 4.23 | **0.009** | 4.87 | **0.001** | 4.45 | **0.001** | 2.29 | **0.008** |
| K6/3189 | 0.15 | 0.871 | 1.71 | **0.001** | −1.25[c] | **0.001** | 2.55 | **0.001** | 3.87 | **0.014** | 2.76 | **0.038** | 1.32 | **0.012** | 2.73 | **0.003** |
| K7/2868 | −0.64[c] | 0.342 | 1.67 | **0.031** | −1.52[c] | **0.003** | 3.88 | **<0.001** | 2.11 | **0.019** | 4.05 | **0.001** | 0.58 | 0.305 | 2.48 | **0.031** |
| K8/3342 | 0.90 | 0.076 | 2.19 | **0.002** | 1.21 | 0.083 | 0.36 | 0.541 | 1.20 | **0.024** | 2.55 | **0.002** | 1.58 | 0.054 | 3.22 | **0.005** |
| E1/1098 | −3.72[c] | **0.002** | −0.29[c] | 0.495 | −0.43[c] | 0.241 | −0.28[c] | 0.612 | 0.45 | 0.370 | 3.72 | **0.001** | 2.81 | **0.001** | 3.68 | **0.002** |
| ATCC 25922 | 1.62 | 0.134 | 2.33 | **0.007** | 2.28 | **0.018** | 2.06 | **0.038** | 4.58 | **0.006** | 4.88 | **0.007** | 2.38 | **0.002** | 3.20 | **0.007** |

[a] K, *K. pneumoniae*; E, *E. coli*; TIG, tigecycline; COL, colistin; GEN, gentamicin; MER, meropenem; RIF, rifampin.
[b] A *P* value of <0.05 indicates significance and is shown in bold.
[c] Antagonistic combination.

penem in 88.9% of strains and more effective ($P < 0.05$) in 66.7% of strains after 24 h, and at 48 h it was superior in 88.9% of strains and more effective in 55.5% of strains. The combination of tigecycline with rifampin was superior to tigecycline with colistin in 88.9% of strains and more effective ($P < 0.05$) in 33.3% of strains after 24 h, and at 48 h it was superior in all strains and more effective in 33.3% of strains. Finally, of note, the combination of tigecycline with rifampin was superior to tigecycline with meropenem in all strains and more effective ($P < 0.05$) in 77.8% of strains after 24 h, and at 48 h it was superior in all strains and more

**TABLE 5** Comparison of the efficacies of tigecycline combinations after 24 and 48 h of treatment[a]

| Isolate | TIG + COL vs TIG + GEN | | TIG + COL vs TIG + MER | | TIG + COL vs TIG + RIF | | TIG + GEN vs TIG + MER | | TIG + GEN vs TIG + RIF | | TIG + MER vs TIG + RIF | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Δlog | *P* value[b] | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value | Δlog | *P* value |
| 24 h of treatment | | | | | | | | | | | | |
| K 1/4089 | 1.71 | **0.003** | 1.20 | **0.003** | 3.47 | **0.005** | −0.50 | 0.085 | 1.73 | **0.033** | 2.37 | **0.001** |
| K 2/4179 | 1.70 | 0.127 | −1.19 | 0.202 | −0.07 | 0.933 | −2.88 | **0.004** | −1.76 | 0.081 | 1.12 | 0.308 |
| K 3/3268 | 1.71 | 0.151 | −1.72 | 0.089 | 1.52 | 0.159 | −3.43 | **<0.001** | −0.19 | 0.147 | 3.24 | **0.001** |
| K4/3712 | 0.75 | **0.049** | −1.42 | **0.038** | 0.56 | 0.098 | −2.17 | **0.003** | −0.91 | **0.003** | 1.99 | **0.003** |
| K5/2458 | 1.05 | 0.159 | −0.24 | 0.648 | 1.37 | **0.047** | −1.23 | **0.002** | 0.315 | 0.168 | 1.61 | **0.003** |
| K6/3189 | 1.12 | **0.040** | 0.13 | 0.615 | 0.97 | 0.083 | −0.99 | **0.004** | −0.15 | 0.397 | 0.84 | **0.011** |
| K7/2868 | 0.70 | 0.245 | 0.30 | 0.580 | 1.69 | **0.032** | −0.90 | **0.006** | −1.17 | 0.114 | 1.55 | **0.026** |
| K8/3342 | 0.86 | 0.135 | 0.23 | 0.520 | 0.56 | 0.311 | −0.63 | 0.092 | 1.00 | 0.226 | 0.52 | 0.229 |
| E1/1098 | −0.35 | 0.280 | 0.04 | 0.942 | 0.74 | 0.215 | 0.39 | 0.303 | −0.64 | 0.371 | 1.98 | **0.034** |
| ATCC 25922 | 0.99 | 0.230 | 2.00 | **0.023** | 0.76 | 0.278 | 1.01 | **0.032** | −0.23 | 0.290 | −1.25 | **0.027** |
| 48 h of treatment | | | | | | | | | | | | |
| K 1/4089 | 2.68 | **0.013** | −1.06 | 0.295 | 3.03 | 0.058 | −3.82 | **0.002** | 0.38 | 0.378 | 4.20 | **<0.001** |
| K 2/4179 | 1.82 | **0.045** | −1.70 | 0.078 | 2.40 | **0.003** | −3.60 | **0.016** | 0.59 | 0.285 | 4.25 | **0.003** |
| K 3/3268 | 0.64 | 0.151 | 1.14 | 0.217 | 2.29 | 0.074 | 0.50 | 0.515 | 1.65 | 0.115 | 1.15 | **0.017** |
| K4/3712 | 0.17 | 0.588 | −0.99 | 0.110 | 0.98 | **0.038** | −1.16 | 0.188 | −1.15 | 0.058 | 1.19 | 0.092 |
| K5/2458 | 0.88 | 0.067 | −0.20 | 0.635 | 0.30 | 0.519 | −1.08 | **0.005** | −0.88 | **0.035** | 0.50 | 0.133 |
| K6/3189 | 1.57 | 0.174 | −1.40 | 0.187 | 2.40 | 0.063 | −2.96 | **<0.001** | 0.84 | **0.003** | 3.80 | **<0.001** |
| K7/2868 | 2.31 | **0.023** | −0.87 | 0.166 | 1.98 | **0.041** | −3.18 | **0.002** | 0.36 | 0.711 | 2.76 | **0.033** |
| K8/3342 | 1.29 | 0.068 | 0.31 | 0.694 | 0.96 | 0.133 | −0.98 | 0.170 | 1.46 | 0.137 | 0.87 | 0.132 |
| E1/1098 | 3.42 | **0.003** | 3.28 | **0.005** | 1.27 | 0.208 | −0.14 | 0.612 | −0.79 | 0.165 | 2.79 | **0.009** |
| ATCC 25922 | 0.71 | 0.251 | 0.66 | 0.191 | 0.44 | 0.186 | −0.05 | 0.802 | −0.27 | 0.364 | −0.23 | 0.313 |

[a] K, *K. pneumoniae*; E, *E. coli*; TIG, tigecycline; COL, colistin; GEN, gentamicin; MER, meropenem; RIF, rifampin.
[b] A *P* value of <0.05 indicates significance and is shown in bold.

effective in 66.7% of strains. There was no clear difference between the activities of the two most effective combinations (tigecycline with gentamicin versus tigecycline with rifampin) and no clear difference between the two least active combinations (tigecycline with colistin versus tigecycline with meropenem) at 24 h and 48 h.

## DISCUSSION

Despite the increasing occurrence and the severity of infections due to carbapenemase producers, limited *in vivo* data exist on the efficacies of the available treatment schemes (8, 9). We performed thigh infections with KPC producers and used tigecycline, colistin, meropenem, rifampin, and gentamicin, alone or in tigecycline combinations, to investigate their *in vivo* activities and their applicabilities in the clinical setting.

When tigecycline was used as monotherapy, bactericidal activity was observed already from 24 h and was evident after 48 h of treatment. Bactericidal activity of tigecycline has been also reported previously *in vivo* against extended-spectrum-β-lactamase (ESBL)-producing bacteria but occurred after 72 h of treatment (10). The study reported in reference 10 supports the findings of the present study and is in contrast with previous observations for bacteriostatic antibiotics, such as tetracyclines (27). Rifampin, even exhibiting high MICs, was generally effective, being comparable to tigecycline and superior to colistin, gentamicin, and meropenem up to 24 h, while its activity was somewhat reduced at 48 h. Similar *in vivo* activity of rifampin has been reported previously against carbapenem-resistant *Acinetobacter baumannii* (28). Nevertheless, rifampin monotherapy is not recommended for the treatment of clinical infections (15). Gentamicin, colistin, and meropenem were rather ineffective as single agents, as was also suggested previously (14).

As for the combinations tested in this study, tigecycline combinations with rifampin or gentamicin exhibited a synergistic effect and were the most effective regimens against most strains. These observations support previous preliminary suggestions that tigecycline combined with either rifampin or an aminoglycoside is promising and could be clinically valuable if validated in animal models (8). In contrast, the addition of colistin and meropenem to tigecycline resulted in antagonism in a considerable proportion of strains, although these combinations, frequently used in clinical practice, were still active and clearly superior to colistin and meropenem used as single regimens. It should be noted that *in vitro* antagonism of tigecycline plus colistin was also observed previously against New Delhi metallo-β-lactamase (NDM)-1-producing *Enterobacteriaceae* (29), while a degree of *in vitro* synergy was observed previously against KPC producers but only up to 4 to 8 h of exposure when tigecycline plus colistin was used at 1× to 2× MIC (13). Regarding the combination of tigecycline with meropenem, previous *in vitro* results indicated suboptimal activity (13), while it was also reported to underperform in clinical infections (30). We could speculate based on this evidence that in clinical cases where the empirical combination of tigecycline plus colistin or a carbapenem was used successfully (14), the treatment outcome was mainly due to the efficiency of tigecycline rather than the antimicrobial activity added by the second agent.

In conclusion, it was observed that among the last-resort antibiotics tested as monotherapies, tigecycline exhibited the best activity, followed by rifampin. Among tigecycline combinations, the addition of rifampin improved considerably tigecycline activity, followed by the addition of gentamicin, while colistin and meropenem did not ameliorate particularly or even deteriorate tigecycline activity. We believe that these *in vivo* data, derived from a soft tissue infection model, substantiate the most active combinations and would be of value to clinicians treating infections due to KPC producers.

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