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NOTE

In vitro activity of tigecycline in combination with rifampin, doripenem or ceftazidime against carbapenem-resistant *Klebsiella pneumoniae* bloodstream isolates

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Klebsiella pneumoniae is one of the most significant nosocomial pathogens in healthcare settings. Over the last decade, *K. pneumoniae* producing extended-spectrum β-lactamases (ESBLs) have been prevalent worldwide. ESBL-producing isolates are considered resistant to all penicillins, cephalosporins and aztreonam, and carbapenems are the only β-lactams that still remain consistently active.[1] But owing to the overuse of carbapenems, resistant strains have become endemic in many countries as well. In addition, most carbapenem-resistant *K. pneumoniae* (CR-KP) are resistant to other antimicrobial classes, referred to as multidrug-resistant (MDR) and extensively drug-resistant strains.[2] The lack of active antimicrobials against CR-KP continues to threaten public health.

Tigecycline is the first member of the glycylcycline class with high affinity to bacterial ribosomes and is unaffected by the typical resistance mechanisms of the tetracycline class.[3] It has appealing *in vitro* activity against resistant Gram-negative bacteria including MDR *Acinetobacter baumannii* and carbapenem-resistant *Enterobacteriaceae*.[3] But the concentrations of tigecycline in serum and pulmonary epithelial lining fluid are suboptimal, and long-term use may lead to resistance *in vivo*.[4] Besides, randomized trials have indicated that the single use of tigecycline may confer an increased mortality risk.[5] Therefore, for enhancing antibacterial activity and reducing resistance development, many physicians prefer to use antibiotic combinations that may act synergistically for treating infections caused by CR-KP.

Though a few studies have suggested tigecycline may act synergistically with other anibiotics,[6,7] the synergistic activities between tigecycline and rifampin, doripenem or ceftazidime have not been well evaluated on a large scale. To provide more guidance for rational drug combinations use in the clinic, we examined the *in vitro* activity of tigecycline in combination with the three drugs against CR-KP bloodstream isolates in this study.

Eighty-five CR-KP clinical strains were isolated from different patients with bloodstream infection in three tertiary hospitals between January 2012 and June 2015 in Shandong, China. *Escherichia coli* ATCC25922 was used as reference strains. All strains were identified using the Vitek 2 Compact System (bioMérieux, Marcyl'Étoile, France). Tigecycline standard was purchased from Sigma-Aldrich (St Louis, MO, USA). Rifampin, doripenem and ceftazidime standards were obtained from the National Institute for the Control of Pharmaceutical and Biological Products, China (Beijing, China).

The MIC of all antibiotics was determined by the broth microdilution method based on the Clinical and Laboratory Standard Institute (CLSI) guidelines.[8] The MIC break points of susceptibility for the three antibiotics were: $\leqslant 1\ mg\ l^{-1}$ for tigecycline, doripenem and ceftazidime according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards.[9] There are no susceptibility break points of rifampin for *Enterobacteriaceae* at present.

Synergy between tigecycline and rifampin, doripenem or ceftazidime was assessed using the microtitre plate checkerboard as described previously.[10] In brief, bacterial suspensions at a cell density of $\sim 10^6$ CFU ml^{-1} were prepared in cation-adjusted Mueller–Hinton broth (Becton Dickinson, Franklin Lakes, NJ, USA). Ninety-six-well microtitre plates were added with 100 μl graded concentrations of antibiotics alone and in combination. Then, 100 μl of bacteria suspension were added to the microplates. After mixing with a vortexer, the microplates were incubated for 24 h at 37 °C in ambient air and were visually inspected for turbidity to determine growth.

Synergy was assessed by calculation of the fractional inhibitory concentration index (FICI). The FICI of each strain for a specific drug combination was calculated with the following formula: FICI = (MIC of drug A in combination/MIC of drug A alone)+(MIC of drug B in combination/MIC of drug B alone). The FICI results were interpreted as follows: synergy, FICI $\leqslant 0.5$; additivity, $0.5 < \text{FICI} \leqslant 1$; indifference, $1 < \text{FICI} < 4$; and antagonism, FICI > 4.[11] The results are expressed as percentages and cumulative inhibition ratios (CIRs) of isolates with synergism, additivity, indifference and antagonism.

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Table 1 FICIs of tigecycline combined with rifampin, doripenem or ceftazidime against 85 carbapenem-resistant *K. pneumoniae* clinical strains

		FICI of tigecycline combined with:		
Standard of result judgment	*Interaction*	*Rifampin No. (%)*	*Doripenem No. (%)*	*Ceftazidime No. (%)*
FICI ⩽ 0.5	Synergy	24/85 (28.2%)	10/85 (11.8%)	7/85 (8.2%)
0.5 < FICI ⩽ 1	Additivity	58/85 (68.2%)	49/85 (77.6%)	29/85 (34.1%)
1 < FICI < 4	Indifference	3/85 (3.5%)	9/85 (10.6%)	49/85 (57.7%)
FICI > 4	Antagonism	0 (0)	0 (0)	0 (0)

Abbreviation: FICI, fractional inhibitory concentration index.

By the checkerboard method, the tigecycline–rifampin combination displayed synergistic and additive activity in 28.2 and 68.2% of the tested isolates (Table 1), and the tigecycline–doripenem combination displayed synergistic and additive activity in 11.8 and 77.6% of the tested isolates. The tigecycline–ceftazidime combination displayed indifferent activity in most strains. Antagonism was not observed in the three combinations.

The CIRs of antimicrobials alone and in combination are shown in Figure 1. Only 68.2% strains were susceptible to tigecycline (MIC $\leqslant 1\ mg\,l^{-1}$), and no strains were susceptible to doripenem (MIC $\leqslant 1\ mg\,l^{-1}$) or ceftazidime (MIC $\leqslant 4\ mg\,l^{-1}$). But when tigecycline combined with rifampin, the susceptibility rate of tigecycline increased to 97.6%, and when tigecycline combined with doripenem, the susceptibility rate of tigecycline increased to 87.1%. Besides, when tigecycline were used alone, the percentages of the 85 isolates whose MICs were below half of the tigecycline maximum concentration in serum (C_{max}) were 35.3%, but in the tigecycline–rifampin and tigecycline–doripenem combination, the percentages increased to 81.2 and 68.2%, respectively. In the tigecycline–ceftazidime combination, the curves did not move markedly to the left, suggesting an indifferent effect.

The CR-KP, especially the *Klebsiella Pneumoniae* Carbapenemase (KPC)-producing strains, have now spread throughout the world and become endemic in many regions, as in our hospitals. They were resistant to most commonly used antibiotics, making the currently effective therapeutic options scarce. Severe infections such as bloodstream infection and nosocomial pneumonia caused by CR-KP are often associated with high treatment failure and >50% mortality rate in critically ill patients.[12] At present, clinical studies suggested that combination therapy offered comparative advantages over monotherapy in treating severe CR-KP infections.[13] In this study, we found that the tigecycline–rifampin and tigecycline–doripenem combinations displayed synergistic and additive activity in most tested isolates. And the addition of rifampin and doripenem at concentrations below C_{max} in serum could significantly decrease the MICs of tigecycline. These findings indicated that the two combinations may be promising and clinically valuable options for treating CR-KP infections.

Rifampin, though exhibited high MICs, was generally effective against Gram-negative bacteria.[14] However, rifampin monotherapy is not recommended in clinical practice, because rifampin-resistant mutants appeared shortly after the initiation of treatment.[14] A recent study performed by Michail *et al.*[15] proved that the *in vivo* activity of rifampin was comparable to tigecycline, and even more effective than colistin, gentamicin and meropenem in some KPC-producing *Enterobacteriaceae* strains at 24 h, but at 48 h, rifampin lost a part of its activity. Their study also found the addition of rifampin to tigecycline produced synergistic effect in 6/9 strains,[15] which was consistent with our results.

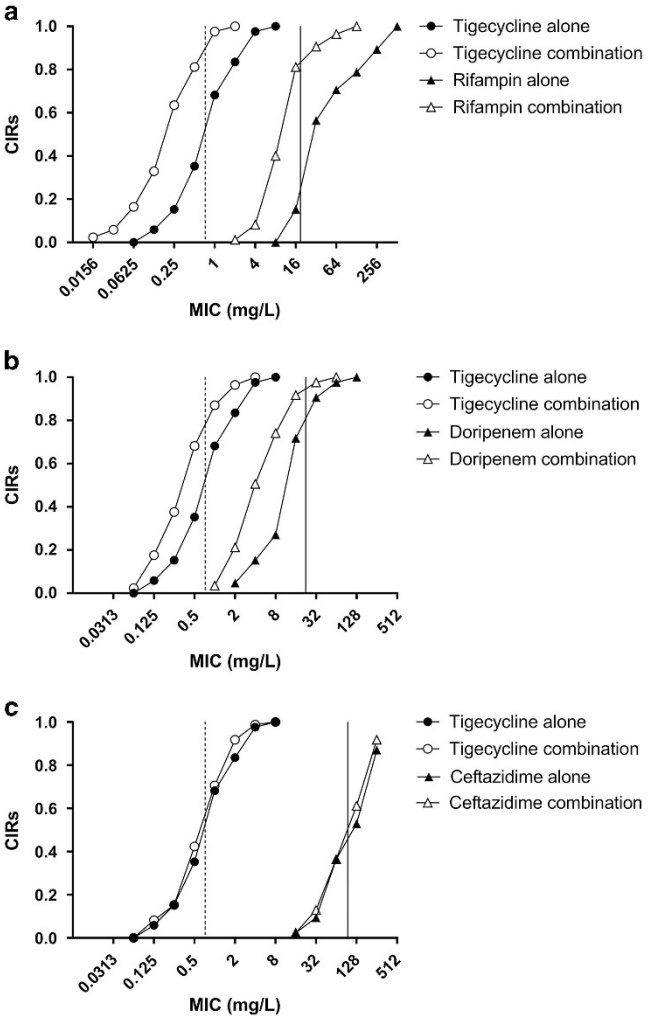

Figure 1 The cumulative inhibition ratios (CIRs) of tigecycline, rifampin, doripenem and ceftazidime alone, and in combination against carbapenem-resistant *Klebsiella pneumoniae* ($n=85$). (**a**) Tigecycline–rifampin combination; (**b**) Tigecycline–doripenem combination; (**c**) Tigecycline–ceftazidime combination. Dashed line, C_{max} in serum of tigecycline (~$0.72\ mg\,l^{-1}$); Solid line, C_{max} in serum of rifampin (~$18.76\ mg\,l^{-1}$), doripenem (~$22.4\ mg\,l^{-1}$) and ceftazidime (~$95.0\ mg\,l^{-1}$).

Though the CR-KP isolates are often resistant to all carbapenems, the question of whether this class of agents has any therapeutic potential against CR-KP infections still remains controversial.[16] Results from animal studies showed that carbapenems retained useful against CR-KP with MICs $\leqslant 4\ mg\,l^{-1}$.[17,18] And accumulating clinical data also suggested that patients with CR-KP infections who received carbapenem-containing combination regimens had significantly lower mortality rates compared with patients who received noncarbapenem-containing regimens, especially in cases where the MIC of the infecting isolate was $\leqslant 4\ mg\,l^{-1}$.[16] In our study, the tigecycline–doripenem combination displayed synergistic or additive activity in 89.4% of the tested isolates. Moreover, before combination, the MIC_{50} of doripenem was $32\ mg\,l^{-1}$; when doripenem combined with tigecycline, the MIC_{50} decreased to $4\ mg\,l^{-1}$. Therefore, tigecycline–doripenem combination may be a robust regimen against infections caused by

CR-KP strains. Considering that both tigecycline and doripenem may bring a risk for gastrointestinal disorders,[3,19] this combination should be carefully used in patients with abnormal liver function.

How tigecycline–rifampin and tigecycline–doripenem combinations act synergistically against CR-KP, or tigecycline–ceftazidime combination act indifferently are still unclear. Tigecycline is a bacteriostatic antibiotic *in vitro* and inhibits bacterial protein synthesis by binding to the 30S ribosomal subunit.[3] We hypothesize that the inhibition of protein synthesis may bring a broad impact on bacteria, for example, the increased permeability of the outer membrane and the decreased quantity of some enzymes. Thereby, the changes of bacteria under tigecycline exposure may allow rifampin more easily to entry into the cell or decrease the degradation ratio of carbapenems but not ceftazidime. Nevertheless, the concrete mechanisms of synergistic activity in these combinations require further investigation.

In summary, our *in vitro* results showed that the tigecycline–rifampin and tigecycline–doripenem combinations have synergistic and additive activity against most CR-KP clinical isolates. The two combinations may be useful in treating CR-KP infections. As the antibiotic concentrations in our study were static, and synergistic activity found in *in vitro* tests may not correlate well with *in vivo* outcomes,[20] these results should be verified with more appropriate methodologies, such as pharmacokinetics/pharmacodynamics models and ideally animal models.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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