Infection and Drug Resistance Dovepress

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The in vitro Activity of Omadacycline Alone and in Combination Against Carbapenem-Resistant *Klebsiella pneumoniae*

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Objective: This study aimed to evaluate the in vitro activity of omadacycline (OMC) and OMC-based combination therapy against carbapenem-resistant *Klebsiella pneumoniae* (CRKP).

Methods: The broth microdilution assay assessed the in vitro susceptibility of CRKP to OMC. The checkerboard assay was performed to evaluate the activity of OMC combined with polymyxin B (PB), amikacin (AN), or meropenem (MEM) against KPC-producing (class A) CRKP strains, and OMC combined with PB, aztreonam (ATM), MEM, or AN against class B and class A plus class B CRKP strains. Synergistic effects of OMC and PB were further evaluated by time-kill assays in the KPC-producing CRKP strains.

Results: Broth microdilution assays revealed a notable variation in susceptibility between KPC-producing and class B CRKP strains, with MIC50/90 of 32/32 mg/L and 0.5/8 mg/L, respectively. Although KPC-producing CRKP strains were resistant to OMC, a synergistic effect was observed in 37.5% of KPC-producing CRKP strains when OMC was combined with PB. In the nine KPC-producing CRKP strains, time-kill assays found that cell densities of six strains (66.7%) decreased by 3.61 ± 0.23 $\log_{10}$ CFU/mL compared to the initial inoculum after 2 hours of PB exposure. The cell densities further decreased by an average of 2.38 ± 0.23 $\log_{10}$ CFU/mL when the six strains were exposed to OMC plus PB, confirming their potent synergism.

Conclusion: OMC monotherapy is ineffective against KPC-producing CRKP strains, but OMC plus PB has a potent synergistic effect on them, suggesting that OMC plus PB is the preferred combination therapy against KPC-producing CRKP in vitro.

Keywords: *Klebsiella pneumoniae*, carbapenem resistance, omadacycline, polymyxin B, antibiotic combination treatment, time-kill test

Introduction

Klebsiella pneumoniae is an opportunistic Gram-negative pathogen that causes many healthcare-associated infections. The rapid spread of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) has become a significant public health concern since the discovery of carbapenemase, creating a severe health burden.[1] High morbidity and mortality combined with insufficient treatment options have led to the recognition of CRKP as a pathogen of critical threat in the global priority list for developing new antibiotics by the World Health Organization in 2017.[2,3] In China, CRKP is among the top five bacterial species present in clinical isolates from hospitals, and the Antimicrobial Surveillance Network in China (CHINET) indicates that clinical isolates of CRKP have increased in prevalence from 3% in 2005 to 21.9% in 2022.[4]

Ceftazidime-avibactam, meropenem (MEM)-vaborbactam, and imipenem-cilastatin-relebactam are recommended as the preferred treatment options for CRKP infection by the Infectious Diseases Society of America.[5] However, there has

Received: 7 October 2024
Accepted: 20 December 2024
Published: 23 December 2024

been a delay in the launch of novel antimicrobial agents in China, and rapidly developing resistance to ceftazidime-avibactam further limits the activity of antimicrobial treatment.[6,7] Therefore, exploring the optimal combination of currently available antimicrobial agents is urgent to maximize efficacy and minimize toxicity in treatment.[8]

Omadacycline (OMC) is the latest generation of tetracycline-class antibiotics with structural modifications at C7 and C9. These modifications allow OMC to effectively combat the most common forms of tetracycline resistance, such as TetK and TetB efflux pumps, as well as TetM and TetO-based ribosome protection mechanisms.[9]

OMC has the advantages of a large volume of distribution in vivo, a low plasma protein-binding rate, and no requirement for dose adjustment in specific populations, making it an ideal tetracycline derivative for treating CRKP infection.[10] However, data on the in vitro susceptibility of CRKP isolates to OMC remains limited. Therefore, we intend to evaluate the in vitro antibacterial efficacy of OMC monotherapy against class A, class B, and class A plus class B CRKP strains, and assess the optimal combination regimens based on OMC against these CRKP strains.

Materials and Methods

Strain Isolation

From March, 18th, 2019 to August 30th, 2022, 53 CRKP isolates were collected from Shanghai Tenth People's Hospital, Children's Hospital of Fudan University, and Shanghai Children's Medical Center (Supplementary Table 1). These strains were identified using a VITEK-MS automatic microbiological analyzer (bioMérieux, Marcy l'Etoile, France) following the standard protocol and referencing the VITEK MS IVD KB V3.2 database. The carbapenemase type of CRKP strains was determined using the NG-test®CARBA5 Carbapenemase Assay Kit (Fosun Diagnostic Technology, Shanghai, China). All isolates were stored at −80°C in a MicrobankTM Storage Box (Pro-Lab Diagnostics, Ontario, Canada) and were sub-cultured twice on Mueller–Hinton (MH) agar plates (Dalian Meilun Biotechnology, Dalian, China) for each experiment.

In vitro Susceptibility Testing

The minimum inhibitory concentrations (MICs) of tigecycline (TIG), polymyxin B (PB), meropenem (MEM), aztreonam (ATM), amikacin (AN), and OMC were determined using the broth microdilution assay and CLSI M07-A9.[11] Analytical grade tigecycline, PB, MEM, ATM, and AN were obtained from Dalian Meilun Biotechnology (Liaoning, China), and analytical grade OMC was obtained from Hanhui Pharmaceuticals (Zhejiang, China). The MICs for tigecycline and OMC were determined based on the United States Food and Drug Administration's recommendations (https://www.fda.gov), whereas the PB MIC was determined using the colistin breakpoints guidance document for 2021 (https://www.eucast.org/). The antibiotic susceptibility results for other antibiotics were interpreted following the CLSI M100 guidelines. *Escherichia coli* ATCC 25922 was used as the quality control strain for each batch.

Molecular Characterization

CRKP strains were tested for the presence of carbapenemase genes via polymerase chain reaction (PCR), and the following primers were used: beta-lactamase New Delhi metallo-beta-lactamase 1 (bla_{NDM}) (F: 5′-GTAGTGCTCAGTGTCGGCAT-3′; R: 5′-GGGCAGTCGCTTCCAACGGT-3′).

Klebsiella pneumoniae carbapenemase (bla_{KPC}) (F: 5′-ATGTCACTGTATCGCCGTC-3′; R: 5′-TTTTCAGAGCCTTACTGCCC-3′), oxacillinase-48 (bla_{OXA-48}) (F:5′-GATCGGATTGGAGAACCAGA-3′; R: 5′-ATTTCTGACCGCATTCCAT-3′), imipenemase (bla_{IMP}) (F: 5′-GGAATAGAGTGGCTTAATTCTC-3′; R: 5′-CCAAACCACTACGTTATC-3′), and Verona imipenemase (bla_{VIM}) (F: 5′-GTGTTTGGTCGCATATCGC-3′; R: 5′-CGCAGCACCAGGATAGAAG-3′). The primer sequences provided by Pasteur (https://bigsdb.pasteur.fr/*klebsiella*/primers-used/) were used to amplify the housekeeping genes *rpoB, gapA, mdH, pgi, phoE, infB*, and *tonB*. The sequences of the isolates were compared against multilocus sequence typing (MLST) databases (https://pubmlst.org/multilocus-sequence-typing) to determine the MLST type of the strains.

Checkerboard Testing

The antibiotics used in our in vitro combination susceptibility tests were selected according to the guideline released by the Society of Bacterial Infection and Resistance of Chinese Medical Association.[12] The checkerboard assay was performed to test the activity of antibiotic combinations. These combinations included OMC plus PB, AN, or MEM, which target KPC-producing CRKP strains. OMC plus PB, ATM, MEM, or AN were used to target class B (NDM, VIM, and IMP) and class A plus class B CRKP strains. Briefly, each antibiotic was prepared in three or four-fold dilutions above and below the MIC using fresh Ca-MH broth. Two different antibiotic dilutions (25μL) were then added to the same well of a 96-well microplate. Subsequently, 3–5 fresh colonies were obtained from the MH agar plates and suspended in sterile saline to create a 0.5 McFarland turbidity inoculum. The bacterial suspension was further diluted 1:100, and 50μL of it was inoculated in each well. After incubating at 35°C for 18–24 h in a CO_2 incubator, the MICs of antibiotic combinations were determined. Fraction inhibitory concentration (FIC) index was used to quantify the interactions between the tested antibiotics and calculated by the following formula: FIC = $A/MIC_A + B/MIC_B$. FIC values ≤ 0.5, 0.5–1, 1–2, and ≥ 2 were interpreted as synergism, additive, indifference, and antagonism, respectively.[13]

Time-Kill Assays

The nine KPC-producing CRKP strains that demonstrated synergistic effects with the combination of OMC and PB (Table 1) were chosen for the time-kill curve analysis. As shown in Table 2, time-kill assays were performed on OMC and PB at the free maximum concentration of the drug in the serum (*f*Cmax) according to the CLSI guideline published in 1999.[14] Colonies on overnight culture plates were selected and diluted to 8 $\log_{10}$ colony forming units (CFU)/mL using sterile saline. The diluted bacterial suspension was added to a flask to ensure a final cell concentration of 6 $\log_{10}$ CFU/mL. Subsequently, 50 μL bacterial suspension samples were sampled at 0, 2, 4, 6, 8, 12, and 24 h, serially diluted,

Table 1 The FIC Values of 9 KPC2-Producing K.p Strains with Synergetic Effect

Isolates	MICs of a Single Antibiotic		MICs of Combined Antibiotics		
	OMC	PB	OMC	PB	FIC
453	32	2	8	0.25	0.375
521	16	2	2	0.25	0.25
525	32	2	8	0.25	0.375
530	32	16	8	1	0.3125
533	32	1	4	0.25	0.375
536	32	2	4	0.25	0.25
538	32	2	4	0.5	0.375
541	32	16	4	2	0.25
553	16	16	4	2	0.375

Notes: FIC, MIC, OMC, and PB represent fraction inhibitory concentration, minimal inhibitory concentration, omadacycline and polymyxin B, respectively. FIC values ≤0.5, 0.5–1, 1–2, and ≥2 were interpreted as synergism, additive, indifference, and antagonism, respectively.

Table 2 Representative Antimicrobial Regimens and fCmax Values Simulated for Each Antibiotic in the Time-Kill Assays

Antibiotics	Antimicrobial Regimens	Cmax (mg/L)	Protein Binding (%)	*fCmax* (mg/L)
Omadacycline	100 mg i.v. over 30 min	2.1	21	1.66
Polymyxin B	1.5 mg/kg i.v. over 1 h	6.21	58	2.61

Note: Cmax and *f*Cmax represent maximum concentration of the drug in the serum and free maximum concentration of the drug in the serum, respectively.

and incubated on MH agar. After overnight incubation, the colonies were counted. Bactericidal activity was defined as a 3 $\log_{10}$ CFU/mL decrease (99.9% killing) in the colony count from the initial colony count after 24 h.[14]

Statistical Analysis

The differences in susceptibility to omadacycline among different carbapenemase types of CRKP were analyzed using Fisher's exact test. Colony count results from the time-kill assays were visualized using GraphPad Prism (version 9.5.1).

Results

Resistance Genes in Isolates

Among 53 CRKP isolates obtained from the Shanghai Tenth People's Hospital, Children's Hospital of Fudan University, and Shanghai Children's Medical Center, 24 harbored a class A carbapenem resistance gene ($bla_{\text{KPC-2}}$), 17 had class B carbapenem resistance genes ($bla_{\text{NDM-1}}$, $bla_{\text{NDM-5}}$, $bla_{\text{IMP-4}}$, $bla_{\text{IMP-8}}$, and $bla_{\text{VIM-1}}$), and 12 had both class A and B carbapenem resistance genes (Figure 1).

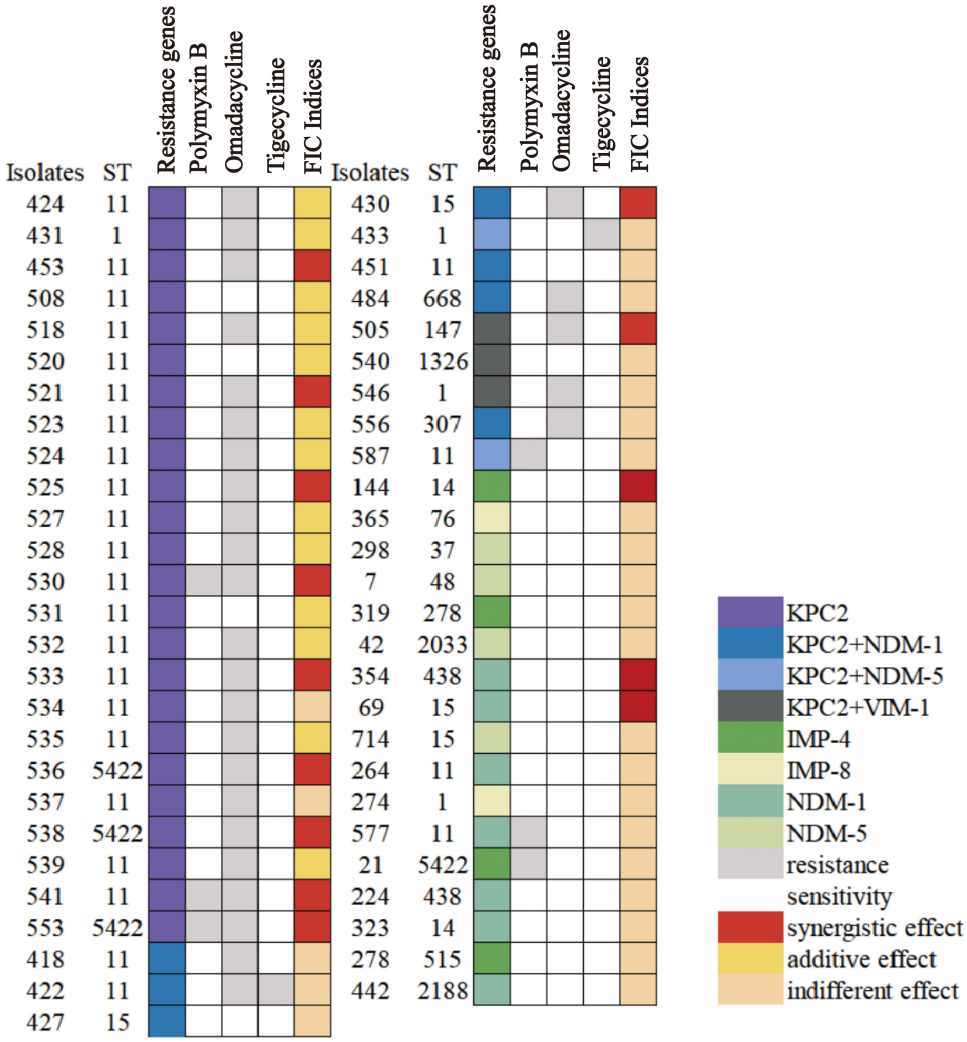

Figure 1 The genotypic characteristics and in vitro susceptibility of 53 CRKP isolates. The first row of the grid represents the resistance genes carried by the CRKP strains, different colors represent different types of resistance genes or combinations. The second, third and fourth row of the grid represent the in vitro susceptibility of CRKP isolates against polymyxin B, omadacycline and tigecycline, respectively, and white and gray represent sensitivity and resistance, respectively. The last row of the grid represents whether omadacycline and polymyxin B have a synergistic effect, with red indicating a synergistic effect, yellow indicating an additive effect and Orange indicating an indifferent effect.

In vitro Susceptibility Testing

The susceptibility of CRKP strains to different antimicrobial agents was determined by in vitro susceptibility testing. As shown in Table 3, the susceptibility of class A and B CRKP to OMC differed significantly ($P<0.01$). All KPC-producing CRKP isolates were resistant to OMC and had an MIC50/90 value at 32 mg/L and 32 mg/L, respectively. Class B CRKP isolates showed good sensitivity to OMC with a high rate of susceptibility (82.3%, 14/17). Only 25% of class A plus class B CRKP isolates showed susceptibility to OMC. No significant difference in tigecycline susceptibility was observed between different carbapenemase types of CRKP isolates. The susceptibility rates for class A, B, and class A plus B CRKP isolates were 83.3% (20/24), 88.2% (15/17), and 83.3% (10/12), respectively. The MIC distributions of antibiotics for 53 CRKP isolates are illustrated in Figure 2. All isolates were resistant to MEM (MIC ranging from 16 to ≥ 256 mg/L). Both class B and class A plus B CRKP isolates showed poor susceptibility to ATM, only 47.1% (8/17) and 8.3% (1/12), respectively.

MLST

Of the 53 CRKP isolates, 17 sequence types (STs) were identified (Figure 1). Among the KPC-producing CRKP isolates, the most common clone was ST11 (n = 20, 83.3%), followed by ST5422 (n = 3, 12.5%), and only one isolate belonged to ST1. The most common clone among class A plus B CRKP isolates was ST11 (n = 4, 33.3%), two isolates belonged to ST15 and ST1 each (16.7%), ST668, ST147, ST1326, and ST307 were also detected. Diverse STs were found in the class B CRKP isolates. Eleven NDM-producing CRKP strains belonged to 8 STs, whereas six IMP-producing CRKP strains belonged to 6 STs.

Checkerboard Test

The checkerboard assay was performed to evaluate the efficacy of different antibiotic combinations in inhibiting the growth of CRKP isolates based on antibiotic interactions. OMC was combined with PB, MEM, or AN against KPC-producing CRKP isolates, and OMC combined with PB, AN, ATM, or MEM was applied for class B, class A plus B CRKP isolates (Table 1 and Supplementary Table 2). When KPC-producing isolates were treated with OMC plus PB, 9

Table 3 The Activity of Omadacycline and Other Antibiotics Against CRKP Isolates

Carbapenemase Types	Antibiotics	MIC			Susceptibility (%)		
		50%	90%	Range	S	I	R
Class A (n=24)	Omadacycline	32	32	8–32	0	12.5	87.5 *
	Polymyxin B	0.5	16	0.5–16	87.5	–	12.5
	Tigecycline	2	4	0.5–4	83.3	16.7	0
	Meropenem	128	256	64–256	0	–	100
Class B (n=17)	Omadacycline	0.5	8	2–8	82.3	17.6	0
	Polymyxin B	0.5	4	0.5–8	88.2	–	11.8
	Tigecycline	0.5	1	0.25–4	88.2	0	11.8
	Meropenem	64	128	16–256	0	–	100
	Aztreonam	32	128	0.25–256	47.1	17.6	35.3
Class A + Class B (n=12)	Omadacycline	16	32	0.5–32	25	16.7	58.3
	Polymyxin B	0.5	1	0.5–16	91.7	–	8.3
	Tigecycline	2	8	0.5–16	83.3	–	16.7
	Meropenem	128	256	16–256	0	–	100
	Aztreonam	128	256	0.5–256	8.3	–	91.7

Notes: The collected 53 CRKP strains were classified into three groups based on the types of carbapenemase they carried. The susceptibility of omadacycline (S: ≤ 4mg/L; I:8mg/L; R: ≥ 16mg/L), polymyxin B sulfate (S: ≤ 2mg/L; R: >2mg/L) and tigecycline (S: ≤ 2mg/L; I:4mg/L; R: ≥ 8m/L) were interpreted according to the standards of FDA, EUCAST and CLSI, respectively. MIC represents minimal inhibitory concentration, * represents $P<0.01$ as compared to the susceptibility rate of class B CRKP isolates to omadacycline.

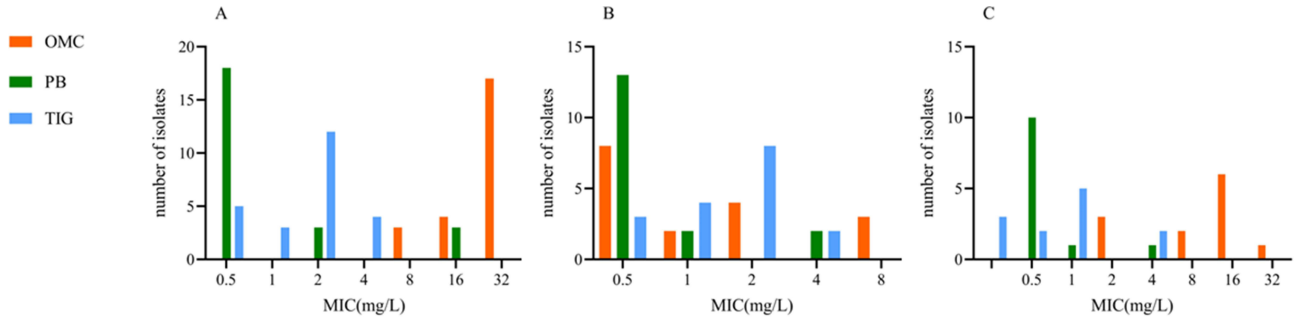

Figure 2 The distribution of MICs for omadacycline, tigecycline and polymyxin B in different carbapenemase types of CRKP strains. (**A–C**) Display the MIC value distribution of OMC, TIG, and PB in class A, class B and class A plus B CRKP isolates, respectively. OMC, TIG, PB and MIC represent omadacycline, tigecycline, polymyxin B and minimal inhibitory concentration, respectively.

cases exhibited the synergistic effect (FIC < 0.5, 37.5%), 13 showed an additive effect (FIC between 0.5 and 1, 54.2%), and the remaining two showed indifference (FIC > 1, 8.3%). However, synergism was shown only in one isolate (4.2%) when OMC plus MEM was applied, an additive effect was observed in three isolates (12.5%), and indifference was observed in the remaining strains (83.3%). In the OMC plus AN group, the additive effect was found in only two strains (8.3%), whereas the other strains (91.7%) were indifferent. Thus, OMC combined with PB exhibited the highest synergism in the KPC-producing CRKP isolates, whereas other antibiotic combinations showed additive or indifferent effects.

In class B CRKP isolates, the OMC plus PB combination showed synergism against three strains (16.7%), and indifference was observed for all strains when OMC plus AN, ATM, or MEM combinations were used. Similarly, OMC plus PB showed synergism against two of the 12 class A plus B CRKP strains (16.7%); when the OMC plus ATM, AN, or MEM combinations were applied, indifference was observed in all 12 isolates.

Time-Kill Curve Assays

Time-kill curve assays were performed on 9 KPC-producing CRKP strains, which showed the synergistic effect of OMC plus PB treatment of the checkerboard test. The 9 CRKP strains were resistant to OMC when comparing the MIC values to the simulated free maximum concentration of the drug in the serum (*f*Cmax) (Table 2),[15,16] with MIC values being 10 to 20-fold higher than the OMC *fCmax* of 1.66 mg/L. Of the 9 strains selected, only three were resistant to PB, and the remaining strains had MIC values lower than the *fCmax* of 2.66 mg/L.

As shown in Figure 3, OMC monotherapy had no bactericidal effect against the KPC-producing CRKP strains, and the cell densities were similar to those of the drug-free control at approximately 8 $\log_{10}$ CFU/mL after 24 h of incubation. In contrast, the PB time-kill curve indicated that the cell densities of six tested strains (66.7%) decreased by 3.61 ± 0.23 $\log_{10}$ CFU/mL after 2 h of antibiotic exposure compared to the initial inoculum (Figure 3A).

Except for KP-530, KP-541, and KP-553, the cell densities of the remaining six strains decreased by an average of 2.38 ± 0.23 $\log_{10}$ CFU/mL after exposure to a combination of OMC and PB for 2 h compared to that obtained using PB alone (4 h for KP-453, Figure 3A). After 8 h of PB and OMC exposure, the bacterial cell count began to increase again, reaching 2.72 ± 1.39 $\log_{10}$ CFU/mL at 12 h, and the bacterial cell count increased to an average of 3.99 ± 1.32 $\log_{10}$ CFU/mL after 24 h. In the time-kill curves of KP-530, KP-541, and KP-553, the cell density remained approximately 8 $\log_{10}$ CFU/mL after 24 h of antibiotic exposure, regardless of PB given alone or combined with OMC (Figure 3B). The average bacterial densities ($\log_{10}$ CFU/mL) of 9 KPC-producing CRKP strains after antibiotic exposure at 2, 4, 6, 8, 12, and 24 hours are shown in Supplementary Table 3.

Discussion

Antibiotic combination therapy is currently the optimal and helpless choice to combat CRKP infection due to the limited supply of novel and effective β-lactamase inhibitors and the increasing resistance to carbapenem.[17–19] Previous studies have suggested that OMC has desirable pharmacokinetic and pharmacodynamic properties. Thus, OMC and OMC-based

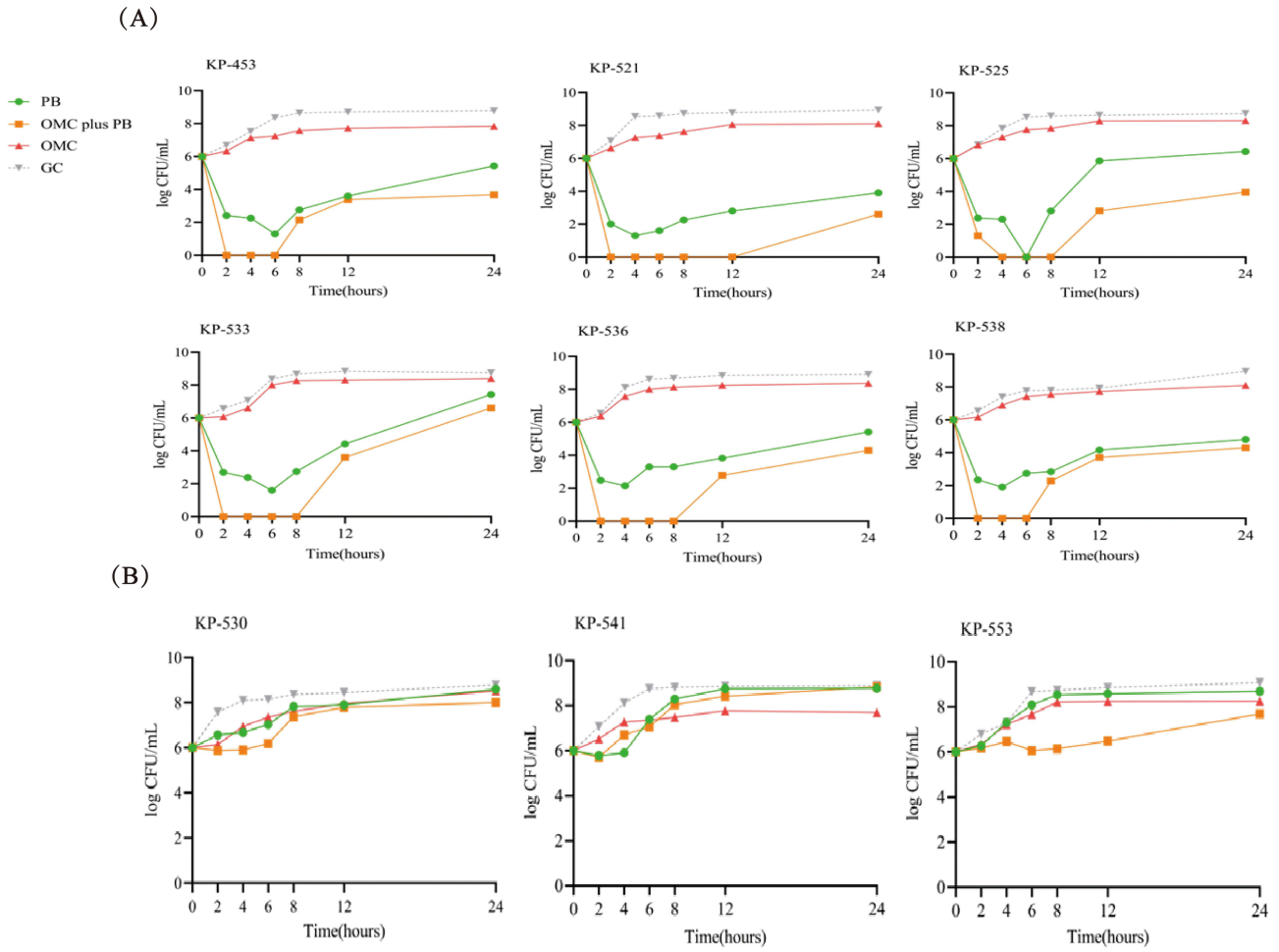

Figure 3 (**A**) The time-kill curves of the 6 polymyxin B sensitive KPC-producing CRKP strains. Time-kill results for OMC, PB and OMC/PB combination against PB sensitive CRKP strains at the free maximum concentration of antibiotic in serum after antibiotics exposure for 2, 4, 6, 8, 12, 24 hours. Curves represent average concentrations from triplicate measurements. PB, OMC, GC and CFU represent polymyxin B, omadacycline, growth control and colony forming units, respectively. (**B**) The time-kill curves of the 3 polymyxin B resistant KPC-producing CRKP strains. Time-kill results for OMC, PB and OMC/PB combination against PB resistant CRKP strains at the free maximum concentration of antibiotic in serum after antibiotics exposure for 2, 4, 6, 8, 12, 24 hours. Curves represent average concentrations from triplicate measurements. PB, OMC, GC and CFU represent polymyxin B, omadacycline, growth control and colony forming units, respectively.

combinations may treat community-acquired bacterial pneumonia or acute skin and soft tissue infections caused by CRKP.[10,20] The present study assessed the in vitro activity of OMC against class A, class B, and class A plus class B CRKP strains. We found that all KPC-producing (class A) CRKP isolates were resistant to OMC alone, and only 25% of class A plus class B CRKP isolates are sensitive, whereas 82.3% of class B CRKP isolates showed good susceptibility. The MIC90 values of class A CRKP strains were as high as 32 mg/L, and all strains were resistant to OMC monotherapy. The high MICs of OMC monotherapy against class A CRKP strains were consistent with a previous study in 2020, but the susceptibility rates were even lower (25% vs 0%).[21] In another in vitro study, CRKP isolates from ICU and non-ICU settings also exhibited relatively low susceptibility rates to omadacycline, with 56.25% and 44.5%, respectively.[22] The reason for the low sensitivity of class A CRKP strains to OMC alone is currently unclear, but may be due to the different abilities of OMC as a substrate for different tetracycline efflux pumps in various carbapenem-producing strains.[23] In addition, OMC has been reported to bind the 70s ribosome to inhibit protein synthesis, but the high-affinity sites and additional low-affinity sites may not be distinguished when OMC is applied.[24] Hence, our in vitro susceptibility tests suggested that OMC monotherapy is not recommended for KPC-producing CRKP infection.

Interestingly, the high resistance rate of OMC monotherapy has not been observed in class B CRKP strains in our study. The susceptibility rate of 17 CRKP strains carrying VIM, NDM, and IMP-encoding genes to OMC is 82.3%, which is slightly higher than the results reported by another OMC susceptibility study, with the susceptibility rates of

bla$_{OXA-48}$-positive and *bla*$_{NDM}$-positive isolates 77.3% and 75.0%, respectively.[25] Furthermore, the susceptibility rates of tigecycline for class A, B, and class A plus B CRKP isolates were 83.3%, 88.2%, and 83.3%, respectively, suggesting that no significant difference in tigecycline susceptibility was found among the CRKP isolates carrying different types of carbapenem resistance genes. Thus, tigecycline monotherapy has a broader sensitivity spectrum against different carbapenemase types of CRKP strains than OMC. Tigecycline may be the preferred choice for patients with CRKP infection who are considering monotherapy; only those infected by class B CRKP can consider monotherapy with either OMC or tigecycline. Besides, the in vitro susceptibility tests also showed that both class B and class A plus B CRKP isolates had poor susceptibility to ATM, only 47.1% (8/17) and 8.3% (1/12), respectively, indicating that the therapeutic effect of ATM on class B and class A plus B CRKP infection is no longer satisfactory.

The disappointing results of OMC monotherapy against KPC-producing CRKP strains have prompted us to consider the possibility of OMC-based antibiotic combination therapy. OMC plus PB is the preferred treatment regimens among all OMC-based antibiotic combinations, as the checkerboard test indicated that OMC combination PB has a synergistic rate of 37.5% on class A CRKP strains. The time-kill assays further proved the bactericidal activity of the PB plus OMC combination against class A CRKP isolates, because the cell densities of the selected KPC-producing CRKP strains (6/9; 66.7%) reached 0 $\log_{10}$ CFU/mL compared to that of the initial inoculum after exposure to PB plus OMC for 2 h. These results suggested that PB combined with OMC could be recommended for class A CRKP infection. The synergistic effects of PB-based antibiotic combinations (with tigecycline, fosfomycin, and AN) against CRKP strains have been previously reported, evidenced by significantly enhanced bactericidal activity and reduced emergence of resistant strains.[8] Meanwhile, the benefits of PB combination therapy for carbapenem-resistant bacterial infection may outweigh any potential harm compared to PB alone, with lower mortality rates, treatment failure, and eradication failure.[12] Moreover, PB-based combinations can also prevent the emergence of resistant subpopulations.[26]

For the mechanisms underlying the synergistic effect of PB plus OMC combination, previous investigation discovered that the interactions between cationic polymyxin molecules and the anionic lipopolysaccharide of the outer membrane represent a crucial initial step in the bactericidal action of PB.[27] After disrupting the outer membrane, the combination of antibiotics can enter bacterial cells.[28] However, the reaction between polymyxin molecules and capsules in *Klebsiella pneumoniae* is more significant than in other bacterial species. The anionic bacterial capsule polysaccharide may reduce potential interactions between cationic polymyxin molecules and the anionic lipopolysaccharide of the outer membrane, contributing to a decrease in the maximal effect against *Klebsiella pneumoniae*.[27,29] The latter may partly explain why there was no synergistic effect of PB plus OMC combination in some KPC-producing CRKP strains. Furthermore, the international consensus guidelines for using polymyxins recommends an area under the curve of approximately 50 mg·h/L as the target concentration.[30] To balance the pros and cons of a high concentration of PB, a limited target PB concentration may also contribute to the failure of combined anti-infection treatment.

Indeed, this study had a few limitations. For example, the time-kill tests performed to evaluate bactericidal activity may not correlate well with in vivo studies, further validation is required in pharmacokinetic/pharmacodynamic models and in vivo infection models. Besides, the mechanism underlying the differential susceptibility of different types of CRKP strains to OMC has not been thoroughly elucidated.

In conclusion, the in vitro susceptibility rate of different carbapenemase types of CRKP isolates to OMC differed considerably, only class B CRKP strains showed good efficacy on OMC monotherapy. The synergistic effect of PB plus OMC found in this study suggested that OMC combined with PB can be considered in patients with KPC-producing CRKP infection. Further genomic investigations are needed to reveal the precise mechanisms that account for the differential susceptibility of class A and class B CRKP to OMC.

Ethics Approval and Informed Consent

The clinical strains utilized in this study were procured from individuals admitted to the Shanghai Tenth People's Hospital. These isolates were collected as a standard procedural measure when performing fluid cultures from infection sites in patients with potential infections. Incorporating these clinical isolates to conduct in vitro susceptibility trials has been approved by the Ethics Committee of Shanghai Tenth People's Hospital under the ethics approval code SHSY-IEC-4.1/18-74/01. The execution of this research was strictly in alignment with the principles stated in the Declaration of Helsinki.

Acknowledgments

We thank Hanhui Pharmaceuticals Company (Zhejiang, China) for providing analytical grade omadacycline.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was supported by the Clinical Research Project of the Shanghai Municipal Health Commission (No. 202340254) and Shanghai Hospital Development Center Foundation (No. SHDC22023234).

Disclosure

The authors report no conflicts of interest in this work.

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