

# Analysis of the Clinical Effect of Combined Drug Susceptibility to Guide Medication for Carbapenem-Resistant *Klebsiella pneumoniae* Patients Based on the Kirby–Bauer Disk Diffusion Method

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**Objective:** To evaluate the in-vitro antibacterial activity of drug combinations against carbapenem-resistant *Klebsiella pneumoniae* (CRKP) and to explore the guiding significance of the combined drug susceptibility results for determining the clinical efficacy in patients with CRKP infection.

**Methods:** Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method. The clinical data of CRKP-infected patients and the drug susceptibility results of sample cultures were gathered and retrospectively analyzed.

**Results:** All 16 CRKP patients had underlying diseases, of which the bloodstream infection was the most common one. Intensive care unit admission history, invasive operation history, and poor nutritional status were recorded to be the high-risk factors. The in-vitro drug susceptibility results indicated that CRKP exhibited 100%, 75.0%, and 66.7% susceptibilities to tigecycline, polymyxin, and ceftazidime/avibatan, respectively. In case of two-drug combinations, polymyxin+tigecycline, ceftazidime avibatan+tigecycline or (aztreonam, polymyxin B, fosfomycin), fosfomycin+polymyxin, imipenem+tigecycline, and fosfomycin+tigecycline exhibited 100%, 87.5% (81.3%, 75.0%, 75.0%), 68.8%, 68.8%, and 62.5%, respectively, synergistic and/or cumulative antibacterial effects. Three-drug combinations such as imipenem+tigecycline+polymyxin, imipenem+fosfomycin+tigecycline, imipenem+fosfomycin+polymyxin, and ceftazidime avibatan+polymyxin+fosfomycin demonstrated 75.0%, 68.8%, 62.5%, and 62.5% synergistic effects, respectively. The clinical efficacy results revealed that the combination of imipenem+tigecycline+fosfomycin showed the best results, followed by meropenem+fosfomycin, imipenem+tigecycline, ceftazidime avibatan, and ceftazidime+amikacin.

**Conclusion:** The combined drug susceptibility results can facilitate guidance of the adjustment of antibacterial drug treatment regimens in patients with CRKP infection. For controlling the CRKP infection, it was found that treatment with carbapenems or ceftazidime avibatan demonstrated better antibacterial activity when combined with tigecycline and/or fosfomycin and/or polymyxin B.

**Keywords:** carbapenem-resistant *Klebsiella pneumoniae*, infection, combined drug susceptibility, Kirby-Bauer disk diffusion method, clinical effect

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## Introduction

*Klebsiella pneumoniae* is a significant member of the enterobacteriaceae family, which is responsible for causing various infections such as respiratory tract infections, urinary tract infections, blood infections, surgical site infections, and

catheter-related infections.<sup>1</sup> Carbapenems are used as the last resort in antimicrobial therapy, especially in cases where extended-spectrum  $\beta$ -lactamase-producing organisms are involved.<sup>2</sup> However, with the indiscriminate use of antibiotics, infection caused by carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is becoming an increasingly serious global problem that is associated with significant morbidity and mortality.<sup>3</sup> CRKP are known to be resistant to multiple antibiotic classes. Presently, only a few drugs such as polymyxins, tigecycline, aminoglycosides, fosfomycin, ceftazidime-avibatan, imipenem-cilastatin-relebactam, and meropenem-vaborbactam exhibit favorable in-vitro activity against CRKP.<sup>4,5</sup> Nonetheless, resistance to polymyxins and tigecycline has been increasingly reported.<sup>6</sup> The World Health Organization (WHO) has classified CRKP as one of the critical priority pathogens requiring urgent research for the development of novel and effective antibiotic therapies.<sup>7</sup> China Antimicrobial Surveillance Network (CHINET) resistance monitoring has also shown that the populations of CRKP are increasing every year.<sup>8</sup> Hence, this limitation in treatment options amplifies the need for effective antibiotics. New drug development is an extensive process, and a study has shown that combination therapy is associated with lower mortality rather than monotherapy among antibiotic-treated CRKP-infected patients.<sup>3</sup> Hence, we screened combinations of antibiotics to study the antibacterial activity against clinical isolates of CRKP in-vitro and provide real-time drug susceptibility results to optimize the antimicrobial therapy for CRKP-infected patients. In our study, antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method, and the results were expressed as susceptible, intermediate, or resistant according to Clinical and Laboratory Standards Institute (CLSI) guidelines.<sup>9</sup> Our research provides further support for the application of combination therapy in treating CRKP infections.

## Materials and Methods

### Specimen Collection

Xinhua Hospital affiliated to the Shanghai Jiaotong University School of Medicine with approximately 2600 licensed beds is a tertiary-care center located in Shanghai. This prospective study analyzed all infected CRKP cases that were identified between March 2020 and August 2020 at Xinhua Hospital, which amounted to a total of 16 cases. All samples were tested for single and combined drug

susceptibility, and the options for CRKP clinical treatment were provided within 48 h. The clinical data of CRKP-infected patients and the drug susceptibility results of the sample cultures were collected.

This study was conducted as per the Good Clinical Practice guidelines and ethical principles that have their origin in the Declaration of Helsinki. The research was approved by the Ethics Committee of Xinhua Hospital affiliated to Shanghai Jiaotong University School of Medicine and was registered at the China Clinical Trial Registry. Signed informed consent was obtained from each subject.

### Bacterial Strains and Antimicrobial Susceptibility Testing

All the CRKP isolates were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS; Brooke Dalton, Germany), and the minimum inhibitory concentrations (MICs) of antibiotics were determined using the Vitek 2 system and the AST-GN card (bioMérieux, France). The Kirby–Bauer disk diffusion method was employed as a supplementary susceptibility test. Carbapenem resistance of CRKP was defined as a minimum inhibitory concentration of  $\geq 4$  mg/L for meropenem/imipenem or  $\geq 2$  mg/L for ertapenem according to the CLSI-2018 criteria. *Escherichia coli* ATCC 25,922, *Klebsiella pneumoniae* ATCC700603, and *Pseudomonas aeruginosa* ATCC27853 were employed as controls for antimicrobial susceptibility testing.

An antibiotic disc was placed at an appropriate distance on Mueller-Hinton Agar (MHA) plates inoculated with a bacterial suspension of 0.5 McFarland turbidity standards, followed by overnight incubation for 18–24 h at 37°C. The antibiotic disks used in this study included the following: amikacin (AK: 30  $\mu$ g), ciprofloxacin (CIP: 5  $\mu$ g), ceftriaxone (CRO: 30  $\mu$ g), cefotaxime (CTX: 30  $\mu$ g), cefuroxime (ROX: 30  $\mu$ g), cefepime (FEP: 30  $\mu$ g), imipenem (IPM: 10  $\mu$ g), meropenem (MEM: 10  $\mu$ g), sulfamethoxazole-trimethoprim (SXT: 3.75/1.25  $\mu$ g), piperacillin-tazobactam (TZP: 100/10  $\mu$ g), ceftazidime (CAZ: 30  $\mu$ g), cefmetazole (CMZ: 30  $\mu$ g), cefoperazone-sulbactam (SCF: 105  $\mu$ g), gentamicin (GEN: 10  $\mu$ g), levofloxacin (LEV: 5  $\mu$ g), ampicillin-sulbactam (SAM: 10/10  $\mu$ g), tigecycline (TGC: 15  $\mu$ g), polymyxin B (PB: 300  $\mu$ g), ceftazidime avibatan (CZA: 50  $\mu$ g), ceftazidime-clavulanic acid (CAC: 30/10  $\mu$ g), aztreonam (ATM: 30  $\mu$ g), amoxicillin-clavulanic acid (AMC: 20/10  $\mu$ g),

fosfomycin (FOT: 200 µg). The antibiotic disks used were sourced from Abtek Biologicals Ltd. (Liverpool, United Kingdom).

Abbreviations note: AK amikacin, CIP ciprofloxacin, CRO ceftriaxone, CTX cefotaxime, ROX cefuroxime, FEP cefepime, IPM imipenem, MEM meropenem, SXT sulfamethoxazole-trimethoprim, TZP piperacillin-tazobactam, CAZ ceftazidime, CMZ cefmetazole, SCF cefoperazone-sulbactam, GEN gentamicin, LEV levofloxacin, SAM ampicillin-sulbactam, TGC tigecycline, PB polymyxin B, CZA ceftazidime avibatan, CAC ceftazidime-clavulanic acid, ATM aztreonam, AMC amoxicillin-clavulanic acid, FOT fosfomycin.

## Combination Disk Test

Combined drug susceptibility testing was performed using the Kirby–Bauer disk diffusion method. The distance between the center points of two pairs of drug-sensitive disks was 24 mm, and any increase in or distortion of the inhibition zone was considered as a positive result. We divided the drug susceptibility results into synergistic, cumulative, irrelevant, and antagonistic effects according to CLSI guidelines and based on the literature reports.<sup>10,11</sup>

## Clinical Materials

To identify the risk factors in the development of CRKP infection, the following data were recorded: demographics (sex and age), comorbidities, sample source, infection site, hospital or ICU admission in the past 30 days, history of invasive operation (such as surgery, deep vein catheterization, endotracheal intubation, and nasogastric tube), antibiotic exposure history, infection degree, nutritional status, and immune functional status.<sup>12</sup> Furthermore, to assess the clinical efficacy for combined drug susceptibility guidance, we recorded the in-vitro drug susceptibility results of specimens, antibacterial agents used throughout the hospitalization, and patient outcomes (improvement or death).

## Statistical Analysis

Statistical analyses were performed using SPSS software (ver. 18.0; SPSS Inc., USA). Mean and standard deviation were used to describe the continuous variables. Proportions and the actual numbers were used to describe the frequency outputs for categorical variables. The data were presented in the form of tables.

## Results

### Demographics of the Study Population

The distribution of the 16 patients with CRKP infection among the various clinical departments was as follows: 3 cases in the respiratory intensive care unit (RICU), 3 cases in the neonatal ward (of which 1 case was in the intensive care unit), 3 cases in the surgical intensive care unit, 2 cases in the pediatric intensive care unit, 2 cases in the general surgical ward, 1 case in the pediatric kidney ward, 1 case in the cardiovascular ward, and 1 case in the hematology ward. There were 11 sites of blood, 1 site of sputum, 1 site of cerebrospinal fluid, 1 site of pleural fluid, and 1 site of bile collection. Of the 16 patients, 12 were men (75%) and 4 were women (25%). Ten of the 16 patients were adults (age: 18–87 years, mean age: 68.2 ± 20.9 years) and 6 were children (age: 2–12 months, mean age: 5.2 ± 4.0 months).

### Antimicrobial Susceptibility of CRKP

The 16 CRKP isolates showed high resistance to almost all the drugs available in the laboratory, especially cephalosporins and carbapenems. The only exceptions were tigecycline, polymyxin B, ceftazidime avibatan, fosfomycin, and amikacin, and the corresponding susceptibility rates were 100%, 93.8%, 75.0%, 50.0%, and 43.8%, respectively (Table 1).

### Combination of Two Drugs Antimicrobial Susceptibility for CRKP in vitro

Examination of the antimicrobial susceptibility of the CRKP isolates to two-drug combinations revealed that polymyxin B and tigecycline combination showed 100% synergistic and/or cumulative antibacterial effects. Second, the combinations of ceftazidime avibatan with tigecycline, aztreonam, polymyxin B, and fosfomycin exerted high synergistic and/or cumulative antibacterial effects of 87.5%, 81.3%, 75.0%, and 75.0%, respectively. Moreover, fosfomycin+polymyxin B (68.8%), imipenem+tigecycline (68.8%), fosfomycin+tigecycline (62.5%), imipenem+polymyxin B (62.5%), and ceftazidime clavulanic acid potassium+polymyxin B (62.5%) also demonstrated appreciable synergistic and/or cumulative antibacterial effects. Meanwhile, the treatment of the CRKP isolates with four times the base dosage of ceftazidime+imipenem combination yielded 62.5% synergistic antibacterial effects. The detailed results are provided in Table 2.

**Table 1** Antimicrobial Susceptibility Distributions of CRKP Isolates

Antibiotics	S	I	R
AK	7 (43.8%)	0	9 (56.2%)
CIP	1 (6.3%)	0	15 (93.7%)
CRO	0	0	16 (100%)
CTX	0	0	16 (100%)
ROX	0	0	16 (100%)
FEP	0	0	16 (100%)
IPM	0	0	16 (100%)
MEM	0	0	16 (100%)
SXT	4 (25.0%)	0	12 (75.0%)
TZP	0	0	16 (100%)
CAZ	0	0	16 (100%)
CMZ	1 (6.3%)	0	15 (93.7%)
SCF	0	0	16 (100%)
CZ	0	0	16 (100%)
GEN	4 (25.0%)	0	12 (75.0%)
LEV	0	0	16 (100%)
SAM	0	0	16 (100%)
TGC	16 (100%)	0	0
PB	15 (93.8%)	1 (6.2%)	0
CZA	12 (75.0%)	3 (18.8%)	1 (6.2%)
FOT	8 (50.0%)	2 (12.5%)	6 (37.5%)
ATM	0	4 (25.0%)	12 (75.0%)

### Combination of 3 Drugs Antimicrobial Susceptibility for CRKP in vitro

The results from studies involving 3-drug combinations indicated that the combination of imipenem, tigecycline, and polymyxin B had the highest (75.0%) synergistic antibacterial activity. The combinations of imipenem+fosfomycin+tigecycline, imipenem+fosfomycin+polymyxin B, and ceftazidime avibatan+polymyxin B+fosfomycin exerted 68.8%, 62.5%, and 62.5% synergistic antibacterial activities, respectively. In addition, imipenem, polymyxin B, and four times ceftazidime clavulanic acid exhibited 56.3% synergistic antibacterial activity. However, ceftazidime avibatan+aztreonam+fosfomycin, meropenem+fosfomycin+tigecycline, and amikacin+fosfomycin+tigecycline had only weak synergistic antibacterial effects of 43.8%, 37.5%, and 37.5%, respectively. The detailed results are provided in Table 3.

### Risk Factors for the Development of CRKP

All the 16 patients with CRKP infection had other complications such as hypertension, diabetes mellitus, solid organ tumor, and anemia. Bloodstream infection was

most commonly encountered, followed by pulmonary disease. It was observed that 75% of the patients had poor nutritional status accompanied by hypoproteinemia, and 62.5% of the patients required parenteral nutrition. In addition, 93.8% of the patients had central venous catheterization, 62.5% had indwelling urinary catheter, and 56.2% had endotracheal intubation and ICU admission history. In light of such findings, a history of invasive operation and ICU admission may be considered as predominant risk factors for CRKP patients. The detailed results are shown in Table 4.

### Therapeutic Effect of Combined Antibacterial Drugs for Patients with CRKP Infection

Among the 16 patients with CRKP infection, 15 (93.7%) had been (or were being) given one or two broad-spectrum antibacterial drugs such as ceftazidime, cefepime, meropenem, imipenem, piperacillin-tazobactam, cefoperazone-sulbactam, tigecycline, amikacin, and aztreonam during the early stage. However, it was inferred that the previously used antimicrobial drugs had a high resistance rate and poor efficacy against CRKP, and hence the CRKP infection did not improve. To accurately and effectively treat CRKP, the antimicrobial therapy regimens were adjusted according to the combined drug susceptibility results obtained from the Kirby–Bauer disk diffusion method. The clinical outcomes showed that the course of antimicrobial treatment for patients with CRKP infection was  $10.7 \pm 4.6$  days.

Furthermore, four of the six children were treated with a combination of meropenem and fosfomycin, and two cases were treated with imipenem+fosfomycin and imipenem+polymyxin B. There were three improvements, one death, and two automatic discharges (giving up treatment). Three of the 10 adult patients were treated with a three-drug combination of imipenem+tigecycline+fosfomycin, and 4 cases were treated with imipenem+tigecycline, ceftazidime+amikacin, ceftazidime avibatan, and meropenem. All the above 7 patients showed improvement after the treatment. Two cases were treated with a combination of meropenem and tigecycline, and both of them involved automatic discharge (giving up treatment). One case was treated with ceftazidime avibatan and amikacin combination but finally died.

Totally, 10 patients (62.5%) exhibited improvement after the treatment regimen was modified according to

**Table 2** Combination of Two Drugs Antimicrobial Susceptibility for CRKP in vitro

Antibiotics	Synergy (%)	Cumulative (%)	Synergy and Cumulative (%)	Irrelevant (%)	Antagonistic
PB+TGC	10(62.5%)	6(37.5%)	16(100%)	0	0
CZA+TGC	12(75.0%)	2(12.5%)	14(87.5%)	2(12.5%)	0
CZA+ATM	13(81.3%)	0	13(81.3%)	3(18.8%)	0
CZA+PB	11(68.8%)	1(6.3%)	12(75.0%)	4(25.0%)	0
CZA+FOT	10(62.5%)	2(12.5%)	12(75.0%)	4(25.0%)	0
IPM +TGC	10(62.5%)	1(6.3%)	11(68.8%)	5(31.3%)	0
FOT+PB	9(56.3%)	2(12.5%)	11(68.8%)	5(31.3%)	0
FOT+TGC	9(56.3%)	1(6.3%)	10(62.5%)	6(37.5%)	0
IPM +PB	10(62.5%)	0	10(62.5%)	6(37.5%)	0
CAZ*4+IPM	10(62.5%)	0	10(62.5%)	6(37.5%)	0
CAC+PB	10(62.5%)	0	10(62.5%)	6(37.5%)	0
CAC+IPM	8(50.0%)	1(6.3%)	9(56.3%)	7(43.8%)	0
IPM +FOT	8(50.0%)	0	8(50.0%)	8(50.0%)	0
CAZ*4+MEM	8(50.0%)	0	8(50.0%)	8(50.0%)	0
AK+TGC	7(43.8%)	0	7(43.8%)	9(56.3%)	0
MEM+PB	7(43.8%)	0	7(43.8%)	9(56.3%)	0
CZA+MEM	7(43.8%)	0	7(43.8%)	9(56.3%)	0
CAZ*4+AK	7(43.8%)	0	7(43.8%)	9(56.3%)	0
MEM+FOT	4(25.0%)	2(12.5%)	6(37.5%)	10(62.5%)	0
PB+AK	5(31.3%)	1(6.3%)	6(37.5%)	10(62.5%)	0
MEM+TGC	6(37.5%)	0	6(37.5%)	10(62.5%)	0
FOT+AK	5(31.3%)	0	5(31.3%)	11(68.8%)	0
IPM +AK	4(25.0%)	0	4(25.0%)	12(75.0%)	0
MEM+AK	4(25.0%)	0	4(25.0%)	12(75.0%)	0
CAC+ATM	4(25.0%)	0	4(25.0%)	12(75.0%)	0
TZP+AK	4(25.0%)	0	4(25.0%)	12(75.0%)	0
IPM +ATM	3(18.8%)	0	3(18.8%)	13(81.3%)	0
IPM +TZP	3(18.8%)	0	3(18.8%)	13(81.3%)	0
IPM +CIP	3(18.8%)	0	3(18.8%)	13(81.3%)	0
TZP+CAZ*4	3(18.8%)	0	3(18.8%)	13(81.3%)	0
MEM+CIP	2(12.5%)	0	2(12.5%)	14(87.5%)	0
MEM+ATM	2(12.5%)	0	2(12.5%)	14(87.5%)	0
CIP+FOT	2(12.5%)	0	2(12.5%)	14(87.5%)	0
ATM+AK	2(12.5%)	0	2(12.5%)	14(87.5%)	0
SCF+AK	2(12.5%)	0	2(12.5%)	14(87.5%)	0
SCF+ATM	2(12.5%)	0	2(12.5%)	14(87.5%)	0
TZP+SCF	1(6.3%)	0	1(6.3%)	15(93.8%)	0
SCF+CIP	0	0	0	16(100.0%)	0
TZP+FOT	0	0	0	16(100.0%)	0

the combined drug susceptibility results. Based on the clinical efficacy treatment results of patients with CRKP infection, it could be inferred that the combination therapy regimens of imipenem+tigecycline+fosfomycin and meropenem+fosfomycin are relatively effective options. Especially in case of pediatric patients for whom antibiotics such as tigecycline, aminoglycoside, and quinolones are not suitable,<sup>13</sup> carbapenems combined with fosfomycin is a relatively safe choice. Meanwhile, the in-vitro antimicrobial susceptibility of CRKP to a combination of

drugs showed that imipenem+tigecycline+fosfomycin combination had 68.8% synergistic antibacterial activity and meropenem+fosfomycin had 37.5% synergistic and/or cumulative antibacterial effects. Other combinations such as imipenem+tigecycline+polymyxin B, polymyxin B +tigecycline, and ceftazidime avibatan+tigecycline also demonstrated high synergistic antibacterial effects. However, the cases were limited, and further evaluation was not possible. The detailed results are presented in Table 5.



**Table 3** Combination of Three Drugs Antimicrobial Susceptibility for CRKP in vitro

Antibiotics	Synergy (%)	Cumulative (%)	Irrelevant (%)	Antagonistic
IPM+TGC+PB	12 (75.0%)	0	4 (25.0%)	0
IPM+FOT+TGC	11 (68.8%)	0	5 (31.3%)	0
IPM+FOT+PB	10 (62.5%)	0	6 (37.5%)	0
CZA+PB+FOT	10 (62.5%)	0	6 (37.5%)	0
IPM+PB+4*CAC	9 (56.3%)	0	7 (43.8%)	0
CZA+ATM+FOT	7 (43.8%)	0	9 (56.3%)	0
MEM+FOT+TGC	6 (37.5%)	0	10 (62.5%)	0
AK+FOT+TGC	6 (37.5%)	0	10 (62.5%)	0
IPM+PB+AK	4 (25.0%)	0	12 (75.0%)	0
IPM+FOT+AK	4 (25.0%)	0	12 (75.0%)	0
MEM+AK+TGC	4 (25.0%)	0	12 (75.0%)	0
MEM+FOT+AK	4 (25.0%)	0	12 (75.0%)	0
IPM+TZP+FOT	3 (18.8%)	0	13 (81.3%)	0
IPM+CIP+TGC	2 (12.5%)	0	14 (87.5%)	0
CAZ*4+AK+SCF	2 (12.5%)	0	14 (87.5%)	0
CAZ*4+AK+IPM	2 (12.5%)	0	14 (87.5%)	0
FOT+ATM+PB	2 (12.5%)	0	14 (87.5%)	0
SCF+ATM+AK	1 (6.3%)	0	15 (93.8%)	0
CAZ*4+TZP+SCF	1 (6.3%)	0	15 (93.8%)	0
MEM+FOT+CIP	1 (6.3%)	0	15 (93.8%)	0
MEM+AK+CIP	1 (6.3%)	0	15 (93.8%)	0
CAZ+ATM+FOT	0	0	16 (100.0%)	0
IPM+SCF+CIP	0	0	16 (100.0%)	0

## Discussion

CRKP has attracted widespread attention owing to the highly limited therapeutic options, and the resistant strains have increased rapidly in recent years.<sup>14</sup> Several observational studies have explored the risk factors for developing CRKP infection, among which undergoing invasive procedures such as deep vein catheterization and tracheostomy and receiving parenteral nutrition exhibited statistical significance.<sup>1</sup> In addition, prior hospitalization, previous antibiotic usage, length of hospitalization, and admission to ICU have been identified as the risk factors for CRKP infection in multiple studies.<sup>1,14,15</sup> A meta-analysis found that patients exposed to major antibiotics such as carbapenems, aminoglycosides, glycopeptides, quinolones, and anti-pseudomonal penicillins have a high risk of acquiring CRKP infection.<sup>1</sup> In this study, all the 16 participating patients had a history of using antimicrobial drugs such as ceftazidime, cefepime, meropenem, imipenem, enzyme inhibitors, tigecycline, amikacin, and aztreonam. In particular, 10 of the 16 patients had been treated with carbapenem antibiotics, which may be closely related to the production of *Klebsiella pneumoniae* carbapenems'

(KPC).<sup>16</sup> Ten cases had poor nutritional status accompanied by hypoproteinemia and were receiving parenteral nutrition. Fifteen patients had central venous catheterization, 10 had indwelling urinary catheter, and 9 had endotracheal intubation and ICU admission history. All the above patients were at high risk according to literature reports.<sup>1,14–16</sup>

A systematic review and meta-analysis found that about one in three CRKP-infected patients receiving antibiotic therapy faced death.<sup>3</sup> Because of the high drug resistance rate, the treatment options for CRKP are limited. No antibiotic regimen could be regarded as the “gold standard” for CRKP infections. In our research, the antimicrobial susceptibility test results of the 16 CRKP isolates indicated high resistance to almost all drugs available in the laboratory, especially cephalosporins and carbapenems. The antibiotics that exhibited the highest vitro activity against CRKP were tigecycline, polymyxin B, ceftazidime avibatan, fosfomycin, and amikacin. These observations are consistent with the findings from previous studies.<sup>17–20</sup> However, in several studies, monotherapy with these agents has been shown to be inferior when compared with combination therapy even though in-vitro

**Table 4** Analysis of the Results of Risk Factors for CRKP

Variables	The Number of Patients That Had These Conditions	Percentage (%)
<b>Comorbidities</b>		
Hypertension	6	37.5
Diabetes mellitus	4	25.0
Solid organ tumor	4	25.0
Anemia	4	25.0
Kidney disease	3	18.8
Cardiac insufficiency	2	12.5
Short bowel syndrome	2	12.5
Multiple organ failure	1	6.3
Respiratory failure	1	6.3
Mucopolysaccharidosis	1	6.3
<b>Infection site</b>		
Bloodstream infection	11	68.7
Pulmonary infection	6	37.5
Central infection	1	6.3
Urinary tract infection	1	6.3
Biliary tract infection	1	6.3
Acute peritonitis	1	6.3
<b>Nutritional status</b>		
ALB<35g/L	12	75.0
ALB≥35g/L	4	25.0
Parenteral nutrition	10	62.5
<b>History of invasive operation</b>		
Central venous Catheterization	15	93.8
Endotracheal intubation	9	56.2
Indwelling urinary catheter	10	62.5
Surgery	4	25.0
<b>ICU admission</b>	9	56.2

testing indicated drug susceptibility.<sup>21</sup> Many studies support the view that combination therapy is associated with a lower mortality rate than monotherapy, and carbapenem with either colistin–polymyxin B or tigecycline was found to be the most commonly used combination.<sup>3,21,22</sup> Based on literature data, we investigated the in-vitro antimicrobial susceptibility of CRKP to combinations of two or three drugs using the Kirby–Bauer disk diffusion method, which is a standard procedure for determining the susceptibility of microbial isolates. When carried out in accordance with the established protocol, reliable results can be obtained and the clinical efficacy of antibiotics can be predicted.<sup>21</sup> Moreover, this method is relatively simple

and quick to operate and can provide results rapidly, which is more in line with the clinical expectations than other methods.

The results of the in-vitro antimicrobial susceptibility of CRKP to two-drug combinations showed that polymyxin B and tigecycline combination exhibited 100% synergistic and/or cumulative antibacterial effects. Other combination such as ceftazidime avibatan and tigecycline (87.5%), ceftazidime avibatan and aztreonam (81.3%), ceftazidime avibatan and polymyxin B (75.0%), ceftazidime avibatan and fosfomycin (75.0%), fosfomycin and polymyxin B (68.8%), imipenem and tigecycline (68.8%), fosfomycin and tigecycline (62.5%), imipenem and polymyxin B (62.5%), and ceftazidime clavulanic acid potassium and polymyxin B (62.5%) also demonstrated substantial synergistic and/or cumulative antibacterial effects. Moreover, the CRKP isolates were investigated with four times dosage ceftazidime and imipenem and four times dosage ceftazidime and amikacin, showing synergistic effects of 62.5% and 43.8%, respectively. The results from these samples could be used to plan an alternative medication regimen for CRKP patients. The in-vitro susceptibility of CRKP to three-drug combinations suggested that the combination of imipenem, tigecycline, and polymyxin B had 75.0% synergistic antibacterial activity. Other combinations such as imipenem+fosfomycin+tigecycline, imipenem+fosfomycin+polymyxin B, and ceftazidime avibatan+polymyxin B+fosfomycin had 68.8%, 62.5%, and 62.5% synergistic antibacterial activities, respectively. Besides, imipenem, polymyxin B, and large doses (4 times) of ceftazidime clavulanic acid possessed 56.3% synergistic antibacterial activity.

Based on the combined in-vitro drug susceptibility results of the CRKP isolates obtained by the Kirby–Bauer disk diffusion method, the clinician adjusted the antimicrobial therapy regimen for the 16 patients with CRKP infection in a timely manner. The average course of the antimicrobial treatment for patients with CRKP infection was  $10.7 \pm 4.6$  days. After the treatment regimen was modified according to the combined drug susceptibility results, 10 patients showed improvement, 3 died, and 3 had automatic discharge (giving up treatment). Four of the six children were treated with a combination therapy of meropenem and fosfomycin, and among them, three showed improvement. Three of the 10 adult patients were treated with a three-drug combination of imipenem +tigecycline+fosfomycin, and all exhibited improvement. Four cases were treated with imipenem+tigecycline,

**Table 5** History of Antimicrobial Use and Clinical Outcome of CRKP Patients

No.	Age	Previous Drug Use	Target Drug for CRKP	Treatment Course for CRKP (d)	Clinical Outcome
Case 1	77Y	MEM, IPM	CAZ+AK	15	1
Case 2	2M	FEP, MEM	MEM+FOT	14	0
Case 3	87Y	ATM	CZA+AK	12	0
Case 4	8M	SCF	MEM+FOT	20	1
Case 5	18Y	CAZ	MEM	5	1
Case 6	3M	CAZ, MEM	MEM+FOT	16	1
Case 7	81Y	TZP, MEM	IMP+TGC+FOT	6	1
Case 8	2M	IPM	IPM+FOT	7	0
Case 9	68Y	MEM, MEM+TGC	CZA	9	1
Case 10	53Y	SCF, CAZ, IPM	IPM+TGC+FOT	7	1
Case 11	82Y	FEP, SCF, MEM	MEM+TGC	4	2
Case 12	4M	/	MEM+FOT	12	1
Case 13	76Y	MEM, IPM+AK	IPM+TGC+FOT	13	1
Case 14	83Y	FEP+TGC	IPM+TGC	11	1
Case 15	57Y	MEM+TGC	MEM+TGC	16	2
Case 16	12M	MEM, TZP+FOT	IPM+PB	5	2

**Notes:** 1 shows as improvement; 2 shows as death; 0 shows as automatic discharge.

ceftazidime+amikacin, ceftazidime avibatan, and meropenem, and they also showed improvement after the treatment. However, the two cases treated with a combination of meropenem and tigecycline were automatic discharge (giving up treatment), and one case treated with ceftazidime avibatan and amikacin combination finally died. In summary, based on the clinical efficacy results of patients with CRKP infection, the combination therapy regimens of imipenem+tigecycline+fosfomycin and meropenem+fosfomycin could be viewed as relatively effective options. Especially for children, carbapenems combined with fosfomycin are a relatively safe choice. The in-vitro antimicrobial susceptibility of CRKP to a combination of imipenem, tigecycline, and fosfomycin showed 68.8% synergistic antibacterial activity, and meropenem+fosfomycin demonstrated 37.5% synergistic and/or cumulative antibacterial effects. Other combinations such as imipenem+tigecycline+polymyxin B, polymyxin B+tigecycline, and ceftazidime avibatan+tigecycline also showed high synergistic antibacterial effects; however, we could not evaluate them further as the cases were limited. Hence, we recommend that the use of combination therapy should depend upon the severity of the illness, infection site, carbapenem resistance mechanism, susceptibility profile of the isolate, and comorbidities of the patient.<sup>17</sup>

## Conclusion

In our research, we examined the in-vitro antimicrobial susceptibility of CRKP isolates to a combination of drugs

to guide the timely adjustment of clinical treatment regimens according to the combined drug susceptibility results. As per the findings, 62.5% of the patients improved after the treatment regimen was modified. However, the sample size was small. To obtain more accurate data, a larger number of samples need to be analyzed in further studies.

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## Disclosure

The authors declare that they have no conflict of interest.

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