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RESEARCH ARTICLE

# Investigation of *in vitro* efficacy of meropenem/polymyxin B and meropenem/fosfomycin combinations against carbapenem resistant *Klebsiella pneumoniae* strains

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**ABSTRACT**

The incidence of infections caused by carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is increasing worldwide, and very limited number of effective antibiotics are available for therapy. In our study, the *in vitro* efficacy of meropenem/polymyxin B and meropenem/fosfomycin combinations against CRKP strains was investigated. The efficiency of meropenem/polymyxin B and meropenem/fosfomycin combinations was tested by checkerboard microdilution and checkerboard agar dilution methods, respectively, against 21 CRKP strains containing major carbapenem resistant genes (7 $bla_{KPC}$, 7 $bla_{OXA-48}$ gene, and 7 $bla_{OXA-48+}$ $bla_{NDM}$), and seven additional CRKP strains without carbapenemase genes.

Among the 28 CRKP strains, the meropenem/polymyxin B combination was synergistic in ten (35.7%), partially synergistic in 12 (42.8%), and indifferent in six (21.4%) isolates. The meropenem/fosfomycin combination was found to be synergistic in three isolates (10.7%), partially synergistic in 20 (71.4%), and indifferent in five (17.8%). In 21 strains containing carbapenem resistance genes, meropenem/polymyxin B and meropenem/fosfomycin combinations exhibited synergistic/partial synergistic effects in 15 (71.4%) and 16 (76.2%) strains, respectively, compared to 100% synergistic/partial synergistic efficiency in both combinations in seven strains free of carbapenemase genes. No antagonistic effect was detected in either combination.

Regardless of presence or absence of carbapenem resistance genes, meropenem/polymyxin B and meropenem/fosfomycin combinations both demonstrated high synergistic and partial synergistic activity against 78.4% and 82.1% of CRKP strains, respectively. Also, they have no antagonistic effects and can be used successfully to prevent therapeutic failure with monotherapy, according to our *in vitro* studies.

**KEYWORDS**

antibiotic combination, fosfomycin, *in vitro*, *Klebsiella pneumoniae*, meropenem, polymyxin B

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## INTRODUCTION

For severe infections caused by extended-spectrum beta-lactamase (ESBL) producing *Klebsiella pneumoniae,* carbapenems are the first-line therapy. Extensive use of carbapenems has led to

the rapid spread of carbapenem-resistant Gram-negative bacilli, especially *K. pneumoniae* strains. This resistance is developed by a deficiency in the antibiotic uptake through porin loss, acquisition of a wide variety of carbapenemase genes harboured on plasmids, that encode enzymes capable of hydrolyzing carbapenems, such as *K. pneumoniae* carbapenemase (KPC), New Delhi metallo-beta-lactamase (NDM), oxacillinase (OXA), imipenemase (IMP), and Verona-encoded metallo-beta-lactamase (VIM) [1].

The monotherapy treatment of infections caused by carbapenem-resistant *K. pneumoniae* (CRKP) strains is mostly difficult, therefore combination therapy is one of the most important option in the treatment of such infections [2]. The use of old antibiotics, which were restricted for different reasons, came to the fore again. For example, polymyxin group antibiotics were removed from the treatment due to their side effects however, they were included again in the combination treatment protocols. Similarly, fosfomycin was removed in the 1970s, but was reused in combination with other antibiotics. The most common use of combined antibiotics is to treat infections caused by carbapenem-resistant Gram-negative bacilli [3].

In our study, we aimed to investigate the *in vitro* effects of meropenem/polymyxin B and meropenem/fosfomycin combinations against CRKP strains.

## MATERIAL AND METHOD

### Collection of carbapenem resistant strains

Twenty-eight CRKP strains isolated from various clinical samples (blood $n = 20$; tracheal aspirate $n = 5$; urine $n = 2$, wound $n = 1$), at Haydarpaşa Numune Training and Research Hospital between December 2020 and August 2021 were included in our study. The identification and antimicrobial susceptibility tests were performed using MALDI-TOF MS (bioMerieux, France) and Vitek-2 (bioMerieux) systems.

The presence of major carbapenem resistance genes ($bla_{KPC}$, $bla_{NDM}$, $bla_{VIM}$, $bla_{IMP}$, $bla_{OXA-51}$, $bla_{OXA23/58}$, $bla_{OXA-48}$) and carbapenemase enzymes have been determined in our laboratory in a previous study [4]. These CRKP strains were included; seven isolates from each of $bla_{KPC}$, $bla_{OXA-48}$, and $bla_{OXA-48+}$ $bla_{NDM}$ and seven strains free of specified genes.

### Determination of minimum inhibitory concentration (MIC)

***Bacterial suspension.*** Bacterial suspension was prepared from overnight cultures of stored strains on tryptic soy agar (TSA, OXOID, UK) medium and adjusted to 0.5 McFarland turbidity (1–1.5 $\times$ $10^8$ cfu $mL^{-1}$) in sterile saline water (0.85%). Then, diluted with cation adjusted Mueller Hinton broth (CAMHB, Becton Dickinson, USA) medium; 10 fold ($10^7$ CFU $mL^{-1}$) for fosfomycin and 200 fold ($5 \times 10^5$ CFU $mL^{-1}$) for meropenem and colistin.

### Preparation of microdilution method

Meropenem (Koçak, Türkiye) and polymyxin B (Koçak, Türkiye) solutions were prepared in CAMHB in the following concentrations: 2–1,024 mg $L^{-1}$ for meropenem and 1–512 mg $L^{-1}$ for polymyxin B, using 96 well microplates (U bottom). All microplates wells were filled with 100 µl of CAMHB, one antibiotic per plate. All plates were stored at −80 °C, to be used within 1–5 days.

For fosfomycin, cation adjusted Mueller Hinton agar (CAMHA) media supplemented with glucose-6-phosphate (25 mg $L^{-1}$) were prepared at 1–4,096 mg $L^{-1}$ concentrations, poured on petri plates (90 mm diameter), and kept at +4 °C to be used within 1–3 days.

### Determination of MIC of meropenem and polymyxin B

Meropenem and polymyxin B microplates were left at room temperature for one hour before use. The first horizontal wells were inoculated with 100 µl of bacterial suspension. CAMHB was inoculated in the eleventh well (the negative control), and the bacterial suspensions were inoculated in the 12th well (the positive control).

### Agar dilution method for determination of fosfomycin MIC

All 14 fosfomycin containing plates were left at room temperature before use, and inoculated with 1–2 µl of bacterial suspension.

All plates and microplates were incubated at 35 °C for 18–24 h. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 strains were used as control strains.

### Evaluation of MICs

The interpretation of meropenem, polymyxin B (epidemiologic cut off) and Fosfomycin MIC values were evaluated according to EUCAST's [5] criteria (Table 1).

### Determination of efficacy of antibiotic combinations

***Checkerboard method with broth microdilution.*** This method was used for the meropenem/polymyxin B combination, according to the MIC values previously obtained.

*Table 1.* Interpretations of meropenem, polymyxin B, and fosfomycin MIC (mg $L^{-1}$) values according to EUCAST criteria

| | Meropenem, mg $L^{-1}$ | | Polymyxin B (mg $L^{-1}$) (epidemiologic cut off) | | Fosfomycin (iv), mg $L^{-1}$ | |
|---|---|---|---|---|---|---|
| | Susceptible | Resistant | Susceptible | Resistant | Susceptible | Resistant |
| *K. pneumoniae* | ≤2 | >8 | ≤2 | >2 | ≤32 | >32 |

Stock solutions of polymyxin B (8 concentrations) and meropenem (9 concentrations) in CAMHB medium were prepared to be equal to four fold MICs. Then, two sets of different concentrations of meropenem/polymyxin B were tested. All vertical wells contained 50 µL of meropenem and horizontal wells were filled with 50 µL of polymyxin B ($\sum$100 µl for each well). The first combination set was serial concentrations of meropenem that varied between 1 mg $L^{-1}$ to 256 mg $L^{-1}$ and polymyxin B initiated from 0.5 mg $L^{-1}$ to 64 mg $L^{-1}$. The second combination set included meropenem which varied between 4 mg $L^{-1}$ to 1,024 mg $L^{-1}$ and polymyxin B between 32 mg $L^{-1}$ to 4096 mg $L^{-1}$. All plates were stored at −80 °C to be used within 1–5 days.

On the working day, microplates were left at room temperature one hour before use. One hundred µL of bacterial suspension ($5.10^5$ CFU $mL^{-1}$) was added to each well. Microplate vertical wells, A10- D10, which contained only bacterial suspensions were positive controls and vertical wells E10- H10 containing only CAMHB, were negative controls. Also, MICs of polymyxin B and meropenem repeated along with the checkerboard method in A11-H11 and A12-H12 microplate columns, respectively. Microdilution plates were incubated at 35 °C for 18–24 h.

***Checkerboard method with agar dilution.*** This method was used for the meropenem/fosfomycin combination, according to the MIC values previously obtained. Stock solutions of fosfomycin (8 concentrations) were prepared to be equal to four fold MICs and for meropenem (9 concentrations) as mentioned in the checkerboard method. Then, two sets of different concentrations of meropenem/polymyxin B were tested. The final concentration of the first combination set was serial concentrations of meropenem that varied between 1 mg $L^{-1}$ to 256 mg $L^{-1}$ and fosfomycin started at 1 mg $L^{-1}$ to 128 mg $L^{-1}$. The final concentration of the second combination set included meropenem which varied between 4 mg $L^{-1}$ to 1,024 mg $L^{-1}$ and fosfomycin which varied between 32 mg $L^{-1}$ to 4,096 mg $L^{-1}$. Then, each combination concentration was added to CAMHA plus G-6-P for the final volume of 150 µl which was previously prepared and kept at 44 ± 1 °C in a bain-marie device. The last mixture was poured into microplate wells. A10-D10 vertical wells contained only CAMHA (positive control), and E10-H10 wells contained only CAMHA (negative control).

MICs of fosfomycin and meropenem were repeated along with the checkerboard agar dilution method in 11 and 12 microplate columns, respectively. All microplates were stored at +4 °C to be used within 1–3 days.

On the working day, 1–2 µL of the previously prepared bacterial suspension ($10^7$ CFU $ml^{-1}$) was inoculated in to all wells (1–2 × $10^4$ CFU) except E10-H10 wells, which there was only CAMHA (negative control). One microplate was used for each strain (Fig. 1). All inoculated plates were incubated at 35 °C for 18–24 h.

## Evaluation of checkerboard method

The MIC values of the antibiotics against the tested bacteria were recorded.

Fractional inhibitory concentration (FIC) values were calculated for both antimicrobials using the following formula [6].

FIC A = MIC value of A antibiotic in combination/MIC value of A antibiotic alone
FIC B = MIC value of B antibiotic in combination/MIC value of B antibiotic alone

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | MEM 1 FOS 1 | MEM 2 FOS 1 | MEM 4 FOS 1 | MEM 8 FOS 1 | MEM 16 FOS 1 | MEM 32 FOS 1 | MEM 64 FOS 1 | MEM 128 FOS 1 | MEM 256 FOS 1 | P.C | FOS | MEM |
| B | MEM 1 FOS 2 | MEM 2 FOS 2 | MEM 4 FOS 2 | MEM 8 FOS 2 | MEM 16 FOS 2 | MEM 32 FOS 2 | MEM 64 FOS 2 | MEM 128 FOS 2 | MEM 256 FOS 2 | P.C | FOS | MEM |
| C | MEM 1 FOS 4 | MEM 2 FOS 4 | MEM 4 FOS 4 | MEM 8 FOS 4 | MEM 16 FOS 4 | MEM 32 FOS 4 | MEM 64 FOS 4 | MEM 128 FOS 4 | MEM 256 FOS 4 | P.C | FOS | MEM |
| D | MEM 1 FOS 8 | MEM 2 FOS 8 | MEM 4 FOS 8 | MEM 8 FOS 8 | MEM 16 FOS 8 | MEM 32 FOS 8 | MEM 64 FOS 8 | MEM 128 FOS 8 | MEM 256 FOS 8 | P.C | FOS | MEM |
| E | MEM 1 FOS 16 | MEM 2 FOS 16 | MEM 4 FOS 16 | MEM 8 FOS 16 | MEM 16 FOS 16 | MEM 32 FOS 16 | MEM 64 FOS 16 | MEM 128 FOS 16 | MEM 256 FOS 16 | N.C | FOS | MEM |
| F | MEM 1 FOS 32 | MEM 2 FOS 32 | MEM 4 FOS 32 | MEM 8 FOS 32 | MEM 16 FOS 32 | MEM 32 FOS 32 | MEM 64 FOS 32 | MEM 128 FOS 32 | MEM 256 FOS 32 | N.C | FOS | MEM |
| G | MEM 1 FOS 64 | MEM 2 FOS 64 | MEM 4 FOS 64 | MEM 8 FOS 64 | MEM 16 FOS 64 | MEM 32 FOS 64 | MEM 64 FOS 64 | MEM 128 FOS 64 | MEM 256 FOS 64 | N.C | FOS | MEM |
| H | MEM 1 FOS 128 | MEM 2 FOS 128 | MEM 4 FOS 128 | MEM 8 FOS 128 | MEM 16 FOS 128 | MEM 32 FOS 128 | MEM 64 FOS 128 | MEM 128 FOS 128 | MEM 256 FOS 128 | N.C | FOS | MEM |

Abbreviation: MEM: meropenem, FOS: fosfomycin, N.C: negative control, P.C: positive control

*Fig. 1.* Schematic view of the checkerboard agar dilution method for testing the efficacy of meropenem/fosfomycin combination on carbapenem resistant *K. pneumoniae* ($n$ = 28) strains

$$\sum \text{FIC} = \text{FICA} + \text{FICB}$$

The ΣFIC index of drug A and B were calculated for all row and column wells that did not display visible growth. The obtained values were summed and divided by the number of wells, and interpreted according to the mean of the ΣFIC index (Table 2).

### Approval of ethical commission

The approval of the Türkiye Minister of Health, Haydarpaşa Numune Training and Research Hospital Clinical Research Ethics Board was obtained (HNEAH- KAEK- 2022/179/3,890).

## RESULTS

Among the 28 CRKP strains tested, 26 were found to be meropenem resistant (MIC, 16–512 mg $L^{-1}$), and two were at the threshold of resistance (MIC, 8 mg $L^{-1}$).

Twenty-seven isolates were polymyxin B resistant (4–256 mg $L^{-1}$), while one isolate was detected at the resistant borderline (MIC, 2 mg $L^{-1}$). Also, 17 strains were resistant (64–2048 mg $L^{-1}$) and 11 strains were found to be susceptible to Fosfomycin (iv) (MIC, ≤32 mg $L^{-1}$).

The MIC values of meropenem, polymyxin B, and fosfomycin in seven isolates with free carbapenemase genes were varied between 64–256 mg $L^{-1}$, 8–256 mg $L^{-1}$, and 8–256 mg $L^{-1}$, respectively, and for 21 isolates with carbapenemase genes, they were determined between 4 to 256 mg $L^{-1}$, 2–256 mg $L^{-1}$, 4–1,024 mg $L^{-1}$, respectively (Table 3).

The checkerboard-broth dilution method revealed that the meropenem/polymyxin B combination had a synergistic effect in ten isolates, a partially synergistic effect in 12 isolates, and an indifference effect in six isolates, with no antagonistic effect on either. The synergy, partial synergism, and indifference effect of meropenem/polymyxin B in each of the seven strains in the group were as follows: three, four, and zero in carbapenemase gene free strains; four, two, and one in $bla^{KPC}$ gene positive strains; two, four, and one in $bla^{OXA-48}$ gene positive strains; and one, two, and four in $bla_{OXA48+}$ $bla_{NDM}$ gene positive strains (Table 3).

The checkerboard agar-dilution method displayed that the meropenem/fosfomycin combination had a synergistic effect in three isolates, a partially synergistic effect in 20 isolates, and an indifference effect in five isolates, with no antagonistic effect on any strain. The synergy, partial synergism, and indifference effects of meropenem/polymyxin in each of the seven strains in the group were as follows: one, six, and zero in carbapenemase gene free strains; zero, six, and one in $bla_{KPC}$ gene positive strains; two, three, and two in $bla_{OXA-48}$ gene positive strains; and zero, five, and two in $bla_{OXA48+}$ $bla_{NDM}$ gene positive strains (Table 3).

*Table 2.* Comment according to ∑FIC index result

| FIC index | Interpretation |
|---|---|
| ≤0.5 | Sinergy |
| >0.5–≤1 | Partial synergy |
| >1–≤4 | Indifference |
| >4 | Antagonist |

## DISCUSSION

In this study, the *in vitro* effects of meropenem/polymyxin B and meropenem/fosfomycin combinations on 28 CRKP strains were investigated. All were producing carbapenemase and distributed as seven strains from each of $bla_{KPC}$, $bla_{OXA48}$, $bla_{OXA48+}$ $bla_{NDM}$, and strains free of carbapenemase genes. The MIC values of the antibiotics were determined in the first stage, and, according to the results obtained, the MIC value ranges that we would use in the combination were planned; however, MIC values were re-studied under the same conditions and on the same plate with the combination study to avoid errors that may arise for technical reasons.

No antagonist efficacy was seen in meropenem/polymyxin B and meropenem/fosfomycin combinations in all isolates. When we compared the efficacy of combinations in strains harbouring carbapenem genes, the efficacy was 71.5% (15/21) in meropenem/polymyxin B combination and increased to 76.2% (16/21) in the meropenem/Fosfomycin combination. The synergy/partial synergy efficacy in both combinations was same (100%) in strains free of carbapenem resistant genes. According to this result and due to no antagonistic effect, combinations studied seem to be a little more effective in strains with no gene detected compared to strains with genes detected. We think that there may be a different mechanism outside the scope of the study that may cause carbapenem resistance, such as efflux pumping, porin loss, or the presence of a different carbapenemase gene/s.

In our study, six *K. pneumoniae* strains resistant to polymyxin B and two strains resistant to fosfomycin were detected in the isolate group ($n = 7$) carrying the $bla_{KPC}$ gene. While no antagonistic effect was found in the combinations of meropenem/polymyxin B and meropenem/fosfomycin in this group, synergy and partial synergy were found to be effective. In the other studies with *K. pneumoniae* strains carrying the KPC gene, Firmo et al. [7], found that combinations of the polymyxin B/meropenem and polymyxin B/amikacin were active against one out of two polymyxin B resistant *K. pneumoniae* co-harbouring $bla_{NDM-1}$, $bla_{KPC-2}$ strains. Sharma et al. [8] found that the polymyxin B/meropenem combination was efficient against nine out of ten $bla_{KPC}$ positive *K. pneumonia* strains by time-kill assay. Maria Souli et al. [9] found synergistic effects of fosfomycin/meropenem and fosfomycin/colistin in 67.4% and 11.8% of clinical isolates of *K. pneumoniae* carrying $bla_{KPC}$, respectively. They explained that all the combinations improved bactericidal activity and decreased fosfomycin resistance. Daikos et al. [10] in a clinical study found that carbapenem containing treatment resulted in the lowest mortality rates against 205 CRPK strains with KPC or VIM producing *K. pneumoniae*. They found additive or synergistic effects of carbapenem in combination with colistin and/or tigecyclin

*Table 3.* MIC values of meropenem, polymyxin B, and fosfomycin, and *in vitro* efficacy of meropenem/polymyxin B and meropenem/fosfomycin combinations in *K. pneumoniae* isolates

| Gen/s | Mem MIC (mg $L^{-1}$) | Poly B MIC (mg $L^{-1}$) | Fos MIC (mg $L^{-1}$) | MEM/Poly B ∑FICI | Mem/Poly B ∑FICI interpretation | Mem/FOS ∑FICI | Mem/Fos ∑FICI interpretation |
|---|---|---|---|---|---|---|---|
| Negative | 128 | 256 | 64 | 0.008 | synergy | 0.803 | partial synergy |
| Negative | 64 | 32 | 64 | 0.1 | synergy | 0.625 | partial synergy |
| Negative | 64 | 32 | 8 | 0.14 | synergy | 0.74 | partial synergy |
| Negative | 64 | 128 | 256 | 0.787 | partial synergy | 0.8037 | partial synergy |
| Negative | 256 | 8 | 32 | 0.516 | partial synergy | 0.344 | synergy |
| Negative | 64 | 256 | 256 | 0.53 | partial synergy | 0.669 | partial synergy |
| Negative | 128 | 64 | 32 | 0.666 | partial synergy | 0.942 | partial synergy |
| KPC | 32 | 2 | 16 | 1.43 | indifference | 0.86 | partial synergy |
| KPC | 32 | 32 | 4 | 0.21 | synergy | 0.646 | partial synergy |
| KPC | 128 | 32 | 32 | 0.664 | partial synergy | 0.843 | partial synergy |
| KPC | 128 | 4 | 4 | 0.465 | synergy | 0.58 | partial synergy |
| KPC | 256 | 64 | 1,024 | 0.394 | synergy | 0.624 | partial synergy |
| KPC | 64 | 16 | 16 | 0.333 | synergy | 1.53 | indifference |
| KPC | 32 | 16 | 256 | 0.89 | partial synergy | 0.684 | partial synergy |
| OXA48 | 64 | 128 | 1,024 | 0.71 | partial synergy | 1.94 | indifference |
| OXA48 | 256 | 128 | 128 | 0.49 | synergy | 1.34 | indifference |
| OXA48 | 8 | 128 | 16 | 2.55 | indifference | 0.434 | synergy |
| OXA48 | 16 | 64 | 32 | 0.723 | partial synergy | 0.535 | partial synergy |
| OXA48 | 16 | 256 | 128 | 0.976 | partial synergy | 0.796 | partial synergy |
| OXA48 | 32 | 80 | 16 | 0.05 | synergy | 0.422 | synergy |
| OXA48 | 8 | 8 | 256 | 0.775 | Partial synergy | 0.504 | partial synergy |
| OXA48+ NDM | 32 | 16 | 256 | 1.098 | indifference | 1.474 | indifference |
| OXA48+ NDM | 16 | 64 | 2,048 | 1.04 | indifference | 0.88 | partial synergy |
| OXA48+ NDM | 32 | 64 | 256 | 0.54 | partial synergy | 0.536 | partial synergy |
| OXA48+ NDM | 64 | 8 | 32 | 0.433 | synergy | 0.853 | partial synergy |
| OXA48+ NDM | 16 | 128 | 256 | 1.94 | indifference | 1.43 | indifference |
| OXA48+ NDM | 16 | 32 | 256 | 1.04 | indifference | 0.536 | partial synergy |
| OXA48+ NDM | 128 | 8 | 64 | 0.54 | partial synergy | 0.70 | partial synergy |

MIC: Minimum inhibitory concentration, Mem: meropenem, Poly B: polymyxin B, FOS: fosfomycin, FICI: fractional inhibitory concentration index.

and/or aminoglycosides. In our study, the combinations of meropenem with polymyxin B or fosfomycin with seven $bla_{KPC}$ positive strains were similar to those in other studies.

Our country, Türkiye, is the country that detected the first OXA-48 positive strain [11]. OXA-48 was the most common gene in strains resistant to carbapenems, and although it is still the predominant carbapenemase enzyme in our country, NDM and KPC are becoming increasingly detected in CRKP isolates [4, 12]. Newer antibiotics such as ceftazidime/avibactam, imipenem/relabactam, and meropenem/vaborbactam are effective against some carbapenemase-positive strains, but none are effective against metallo-beta-lactam-containing strains, nor are meropenem/vaborbactam or imipenem/relabactam-carrying OXA-48 strains [13]. In this situation, antibiotic combinations are an important way to treat infections with $bla_{OXA-48}$ alone or $bla_{OXA-48+}$ $bla_{NDM}$ strains. In our study, no antagonistic effects were found in the isolates carrying the $bla_{OXA-48}$ gene ($n$ = 7) with the combinations of meropenem/polymyxin B and meropenem/fosfomycin. In the same group, indifference effects were found in one strain of the meropenem/polymyxin B combination and in two strains of the meropenem/fosfomycin combination. In the study of Evren et al. [14] the *in vitro* activity (synergy) of meropenem/fosfomycin on 12 positive $bla_{OXA-48}$ *K. pneumoniae* strains were found to be 33% using the checkerboard method, they also found antagonism in one strain. In our study, we found the activity of this combination in solely $bla_{OXA-48}$ and $bla_{OXA-48+}$ $bla_{NDM}$ *K. pneumoniae* strains, two fold higher than that of Evren et al. finding. Sengel et al. [15] found synergy in five strains and indifference in two strains of *K. pneumoniae* carrying the $bla_{OXA-48}$ gene in the combination of meropenem/fosfomycin using the checkerboard method. In the same study, two strains with FICI values of 0.51 and 0.81 in fosfomycin/meropenem combination and in the study of Evren et al. [14], FICI values of six strains (FICI: 0.52–0.76) were evaluated as indifference, while within the scope of our study, these FICI values were accepted as partial synergy.

Sengel et al. [15] reported synergistic effects in the combination of meropenem/fosfomycin in all five $bla_{OXA-48+}$ $bla_{NDM}$ *K. pneumoniae* strains. We found indifference in two strains and partial synergy in five strains, but no synergy or antagonistic effects were detected. Also, MIC values of fosfomycin in our five strains were ≥256 mg $L^{-1}$ and higher

than MICs of four strains from Sengel et al., which might be the reason behind the difference that is reflected in the results of the checkerboard antibiotic combination in two studies.

## CONCLUSION

In the 28 *K. pneumonaie* strains studied, no antagonistic effect was found in combinations of meropenem/polymyxin B and meropenem/fosfomycin.

It was determined that the synergy/partial synergy efficacy percentages of meropenem/polymyxin B and meropenem/fosfomycin against all tested *K. pneumonia* were 78.6% and 82.3%, respectively, regardless of the presence or absence of carbapenem resistance genes.

According to our *in vitro* results, meropenem with polymyxin B and fosfomycin combinations are promising treatments for carbapenem resistant *K. pneumoniae* infections.

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## REFERENCES

1. Xu L, Sun X, Ma X. Systematic review and meta-analysis of mortality of patients infected with carbapenem-resistant *Klebsiella pneumoniae*. Ann Clin Microbiol Antimicrob 2017; 29, 16(1):18: 1–12. https://doi.org/10.1186/s12941-017-0191-3.
2. Durante-Mangoni E, Andini R, Zampino R. Management of carbapenem-resistant Enterobacteriaceae infections. Clin Microbiol Infect 2019; 25(8): 943–50. https://doi.org/10.1016/j.cmi.2019.04.013.
3. Chiotos K, Hayes M, Gerber JS, Tamma PD. Treatment of carbapenem-resistant *Enterobacteriaceae* infections in children. J Pediatr Infect Dis Soc 2020; 28; 9(1): 56–66. Available from: https://doi.org/10.1093/jpids/piz085.
4. Çalık Ş, Kansak N, Aksaray S. Phenotypic detection of carbapenemase production in carbapenem-resistant isolates with the rapid carbapenemase detection method (rCDM). J Microbiol Methods 2022; 200: 1–7. https://doi.org/10.1016/j.mimet.2022.106536.
5. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0, 2022. http://www.eucast.org.
6. JA Hindler and RM Humphrie. Antimicrobial susceptibility testing, pp: 813–1110: Amy L L.clinical microbiology procedures hand book. 2016 4th ed. ASM Press. Washington D C.
7. Firmo EF, Oliveira Júnior JB, Scavuzzi AML, Alves LC, Brayner FA, Veras DL, et al. *In vitro* activity of polymyxin B in combination with meropenem, amikacin and gentamicin against *Klebsiella pneumoniae* clinical isolates co-harbouring aminoglycoside-modifying enzymes, $bla_{NDM-1}$ and $bla_{KPC-2}$. J Glob Antimicrob Resist 2020; 22: 511–4. https://doi.org/10.1016/j.jgar.2020.04.014.
8. Sharma R, Patel S, Abboud C, Diep J, Ly NS, Pogue JM, et al. Polymyxin B in combination with meropenem against carbapenemase-producing *Klebsiella pneumoniae*: pharmacodynamics and morphological changes. Int J Antimicrob Agents 2017; 49(2): 224–32. https://doi.org/10.1016/j.ijantimicag.2016.10.025.
9. Souli M, Galani I, Boukovalas S, Gourgoulis MG, Chryssouli Z, Kanellakopoulou K, et al. *In vitro* interactions of antimicrobial combinations with fosfomycin against KPC-2-producing *Klebsiella pneumoniae* and protection of resistance development. Antimicrob Agents Chemother 2011; 55(5): 2395–7. https://doi.org/10.1128/AAC.01086-10.
10. Daikos GL, Tsaousi S, Tzouvelekis LS, Anyfantis I, Psichogiou M, Argyropoulou A, et al. Carbapenemase-producing *Klebsiella pneumoniae* bloodstream infections: lowering mortality by antibiotic combination schemes and the role of carbapenems. Antimicrob Agents Chemother 2014; 58(4): 2322–8. https://doi.org/10.1128/AAC.02166-13.
11. Poirel L, Héritier C, Tolün V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2004; 48(1): 15–22. https://doi.org/10.1128/AAC.48.1.15-22.2004.
12. Süzük Yıldız S, Şimşek H, Bakkaloğlu Z, Numanoğlu Çevik Y, Hekimoğlu CH, Kılıç S, et al. Ulusal Karbapenemaz Sürveyans ÇalışmaGrubu. Türkiye'de 2019. Yılı İçinde İzole edilen *Escherichia coli* ve Klebsiella pneumoniae İzolatlarında Karbapenemaz epidemiyolojisi [The epidemiology of carbapenemases in *Escherichia coli* and *Klebsiella pneumoniae* isolated in 2019 in Turkey]. Mikrobiyol Bul 2021 Jan; 55(1): 1–16. TurkİSh. https://doi.org/10.5578/mb.20124.
13. Bassetti M, Giacobbe DR, Giamarellou H, Viscoli C, Daikos GL, Dimopoulos G, et al. Critically Ill patients study group of the European society of clinical microbiology and infectious disease (ESCMID); Hellenic society of chemotherapy (HSC) and Società Italiana di Terapia anti infettiva (SITA). Management of KPC-producing *Klebsiella pneumoniae* infections. Clin Microbiol Infect 2018; 24(2): 133–44. https://doi.org/10.1016/j.cmi.2017.08.030.
14. Evren E, Azap OK, Çolakoğlu Ş, Arslan H. *In vitro* activity of fosfomycin in combination with imipenem, meropenem, colistin and tigecycline against OXA 48-positive *Klebsiella pneumoniae* strains. Diagn Microbiol Infect Dis 2013; 76(3): 335–8. https://doi.org/10.1016/j.diagmicrobio.2013.04.004.
15. Erturk Sengel B, Altinkanat Gelmez G, Soyletir G, Korten V. *In vitro* synergistic activity of Fosfomycin in combination with meropenem, amikacin and colistin against OXA-48 and/or NDM-producing *Klebsiella pneumoniae*. J Chemother 2020; 32(5): 237–43 https://doi.org/10.1080/1120009X.2020.1745501.