In vitro evaluation of double-carbapenem combinations against OXA-48-producing Klebsiella pneumoniae isolates using time–kill studies

Irene Galani,* Konstantina Nafplioti, Marianthi Chatzikonstantinou and Maria Souli

Abstract

Purpose. The aim of this study was to evaluate the in vitro activity of double-carbapenem combinations against OXA-48-producing Klebsiella pneumoniae clinical isolates.

Methodology. Double combinations of ertapenem, meropenem and imipenem were evaluated for synergy and bactericidal activity using the time–kill methodology. All antibiotics were tested at 10 mg l⁻¹ and at a sub-inhibitory concentration of 0.5× minimum inhibitory concentration (MIC) for isolates with a carbapenem MIC ≤ 8 mg l⁻¹. Synergy was defined as a ≥2log₁₀ colony-forming units (c.f.u.) ml⁻¹ decrease of viable colonies at 24 h compared to the most active carbapenem alone.

Results. Ten distinct K. pneumoniae clinical isolates were tested. All carried blaOXA-48 and blaCTX-M-15, and exhibited an MIC range of 64–128, 4–32 and 1–32 mg l⁻¹ for ertapenem, meropenem and imipenem, respectively. Out of 48 isolate-combinations, synergy was observed in 9 (18.8 %) and cidal activity was observed in 13 (27.1 %). In vitro synergistic activity was noted for 5 out of 29 (17.2 %) ertapenem-meropenem-, 6 out of 29 (20.7 %) meropenem-imipenem- and 7 out of 38 (18.4 %) imipenem-containing combinations. No combination exhibited antagonism. Bactericidal activity was observed in 7 (24.1 %) ertapenem-, 8 (27.6 %) meropenem- and 11 (28.9 %) imipenem-containing combinations. Among the sub-inhibitory concentration combinations, three (15 %) ertapenem-, four (20 %) meropenem- and three (15 %) imipenem-containing ones showed synergistic interaction.

Conclusion. Dual combinations of carbapenems, including those containing sub-inhibitory concentrations of antibiotics, were synergistic against multidrug-resistant (MDR) and extensively drug-resistant (XDR) K. pneumoniae isolates harbouring blaOXA-48.

INTRODUCTION

Among over 550 class D β-lactamases, also known as oxacillinas (OXAs), OXA-48 is the most prevalent enzyme with carbapenemase activity. OXA-48 was first identified in Klebsiella pneumoniae in Turkey in 2003 [1], and thus far 12 variants of the blaOXA-48 gene have been identified, with OXA-436 being the most recent one [2, 3]. Turkey is one of the main reservoirs of OXA-48-producing K. pneumoniae, but since 2003 the endemic spread of these bacteria has been reported in many countries around the Mediterranean (Morocco, Libya, Egypt and Tunisia), as well as in India. Currently, OXA-48 and its derivatives have been reported worldwide, but with increasing prevalence in Spain, France, Lebanon and Saudi Arabia [4].

Two specific features of the blaOXA-48 gene are worth mentioning. (1) The potential for global dissemination, even in community settings. This is because the gene is usually located on a highly transferable plasmid with a very high conjugation rate that is capable of spreading among various clones, as well as among various species of Enterobacteriaceae. (2) The unique hydrolytic properties of the respective enzyme. It is a weak carbapenemase and spares broad-spectrum cephalosporins; it is thus associated with low-level resistance, unless there is a permeability deficiency or co-production of extended spectrum β-lactamase(s) [ESBL(s)]. For these reasons, OXA-48 has been described as a ‘phantom menace’ [5]. Remarkably, enzyme kinetic analysis shows greater catalytic activity of OXA-48 to imipenem.

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Keywords: imipenem; meropenem; ertapenem; synergy; carbapenemase.

Abbreviations: ERT, ertapenem; ESBL, extended spectrum β-lactamase; IEF, isoelectric focusing; IMP, imipenem; MDR, multidrug resistant; MEM, meropenem; MIC, minimum inhibitory concentration; OXA, oxacillinase; PDR, pan-drug resistant; PFGE, pulsed field gel electrophoresis; XDR, extensively drug resistant.

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(approximately 100-fold) than to 1-β-methyl-carbapenems, meropenem and ertapenem [6].

The treatment of infections caused by OXA-48-producing Enterobacteriaceae remains a challenge and despite high rates of susceptibility to non-β-lactam antibiotics, the mortality associated with bacteremia is as high as 40–50% [7, 8]. Systematic reviews of the available literature favour the use of combination regimens for the treatment of serious infections by carbapenem-resistant Enterobacteriaceae [9], but clinical experience with OXA-48 producers is limited. Furthermore, in vitro studies evaluating the advantage of combination regimens against OXA-48 producers are not as extensive.

The objective of our study was to evaluate the in vitro activity, through time-kill assays, of double-carbapenem combinations against OXA-48-producing K. pneumoniae clinical isolates.

METHODS

Ten distinct OXA-48-producing K. pneumoniae clinical isolates were used in this study, and these were recovered from urine (n=4), blood (n=3), rectal swabs (n=2) and bronchial aspirate (n=1) cultures of single hospitalized patients in four distinct hospitals of the Athens Metropolitan area.

Species identification of isolated bacteria and minimum inhibitory concentration (MIC) determinations were performed using an automated system (the BD Phoenix automated microbiology system; Becton Dickinson Diagnostic Systems, Sparks, MD, USA). Isoelectric focusing (IEF) of sonic extracts on pre-cast polyacrylamide gels with a pH 3–10 gradient (PhastGel IEF 3–9; Amersham Biosciences, Freiburg, Germany) was performed for all isolates. β-lactamasises were detected as red bands after overlaying the gel with filter paper soaked with a 0.25 mM nitrocefin. The presence of β-lactamase-encoding genes was confirmed by PCR using specific primers and sequencing [10].

Carbapenem MICs were further determined by broth microdilution and interpreted per EUCAST breakpoints [11]. Multidrug-resistant (MDR) and extensively drug-resistant (XDR) phenotypes were characterized as previously reported [12].

Genetic relatedness among studied isolates was evaluated with pulse – field gel electrophoresis (PFGE) of chromosomal restriction fragments obtained after SpeI cleavage. Cluster analysis was performed using PFQuest software version 5.1 (Bio-Rad Laboratories, Inc., CA, USA), and a dendrogram was generated from the homology matrix with a coefficient of 1.5% using the unweighted pair-group method using arithmetic averages (UPGMA) to describe the relationships among PFGE profiles.

In vitro interactions of dual combinations of imipenem, meropenem and ertapenem were tested using the time–kill methodology. Tubes containing cation-adjusted Muller–Hinton II broth (Becton Dickinson) and each antibiotic alone or in combination were inoculated with 5×10⁵ colony-forming units (c.f.u.) ml⁻¹ of the studied strain and were quantitatively sub-cultured at 0, 1, 3, 5, 7 and 24 h of incubation for viable colony counts. At the above time intervals, an aliquot of 0.1 ml was removed from each tube, serially diluted (seven ten-fold dilutions) and plated on MacConkey (Becton Dickinson) agar plates. Dilution was expected to minimize any probable antibiotic carry-over effect. The results were expressed as log¹⁰ c.f.u. ml⁻¹. All antibiotics were tested at the concentration of 10 mg l⁻¹, because this represented the mean concentration of the free drug achievable in human serum with conventional dosing [13–15]. For isolates with an imipenem or meropenem MIC≤8 mg l⁻¹, a sub-inhibitory concentration of 0.5× MIC was also tested. A tube without antibiotic was also included in each experiment as a growth control. The lower limit of detection was 1.6 log¹⁰ c.f.u. ml⁻¹.

Synergy was defined as a ≥2log¹⁰ decrease in c.f.u. ml⁻¹ between the combination and the most active single agent at 24 h, with the number of surviving organisms in the presence of the combination being ≥2log¹⁰ c.f.u. ml⁻¹ below the number of organisms in the starting inoculum. Antagonism was defined as a ≥2log¹⁰ increase in c.f.u. ml⁻¹ between the combination and the most active single agent. All other interactions were characterized as indifferent. The bactericidal activity of single antibiotics or combinations was defined as a ≥3log¹⁰ reduction in the c.f.u. ml⁻¹ of the initial inoculum [16, 17].

For statistical analysis, Fisher’s exact test was used to compare the proportions of killing activity in 2×2 tables. A P value of ≤0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The MICs of carbapenems against the studied isolates and their susceptibilities to other antimicrobials are shown in Table 1. Regarding their susceptibility profile, two of our isolates exhibited an MDR and eight an XDR phenotype.

All 10 K. pneumoniae isolates carried blaOXA-48 and bldCTX,M-15. According to the PFGE results, five distinct pulsotypes (A–E) were revealed using the 80% strain similarity threshold. Five isolates assigned to pulsotype C were further divided into two subgroups (C1, C2) showing greater than 80% but less than 90% similarity (Fig. S1, available in the online version of this article). Among the tested isolates, b 6921161 and U 75 exhibited an identical banding pattern, and were assigned as pulsotype A, while isolates 178-GNA and SP 153 also exhibited the same banding pattern as each other, and were assigned as subtype C2. Isolates of the same pulsotype/subtype were included in the study if their susceptibility phenotype differed by one antibiotic class (Table 1).

Out of the 48 isolate combinations tested, synergy was observed in 9 (18.8%) and cidal activity was observed in 13 (27.1%). All other isolate combinations resulted in indifference (Table 1), while no combination exhibited antagonism.
Table 1. Phenotypic and genotypic characteristics of 10 OXA-48-producing *K. pneumoniae* clinical isolates

Interactions of double-carbapenem combinations and differences between the starting inoculum and the number of residual viable colonies (Δlog$_{10}$ c.f.u. ml$^{-1}$) after 24 h of incubation with each combination. ERT, ertapenem; MEM, meropenem; IMP, imipenem; Ind, indifference; Syn, synergy; NA, not applicable.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Clonal type</th>
<th>MIC (mg l$^{-1}$)</th>
<th>Interaction of combination at 24 h</th>
<th>Δlog$_{10}$ c.f.u. ml$^{-1}$ between initial and final inoculum*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ERT MEM IMP</td>
<td>ERT/ MEM† ERT/IMP (10 mg l$^{-1}$)‡ ERT/IMP (0.5 × MIC)‡ MER/IMP (10 mg l$^{-1}$)‡ MER/IMP (0.5 × MIC)‡</td>
<td>ERT/ MEM† ERT/IMP (10 mg l$^{-1}$)‡ ERT/IMP (0.5 × MIC)‡ MER/IMP (10 mg l$^{-1}$)‡ MER/IMP (0.5 × MIC)‡</td>
</tr>
<tr>
<td>b 692161</td>
<td>$§$</td>
<td>A 128 32 4</td>
<td>Ind Ind Ind Ind</td>
<td>3.68 3.70 4.40 4.18 2.80</td>
</tr>
<tr>
<td>U 75$</td>
<td>A 128 16 1</td>
<td>Ind Syn Ind</td>
<td>3.67 −4.00 4.20 −4.00 3.67</td>
<td></td>
</tr>
<tr>
<td>U 184</td>
<td></td>
<td>B 64 16 4</td>
<td>Ind Ind Ind</td>
<td>3.80 3.92 3.80 4.07 4.12</td>
</tr>
<tr>
<td>V 5600</td>
<td>C1 64 64 4</td>
<td>Ind Ind Ind</td>
<td>3.62 4.40 3.70 3.82 3.70</td>
<td></td>
</tr>
<tr>
<td>NT 2§</td>
<td>C1 128 16 2</td>
<td>Ind Ind Ind</td>
<td>3.14 −4.00 4.40 −4.60 3.80</td>
<td></td>
</tr>
<tr>
<td>SP 153</td>
<td></td>
<td>C2 128 32 8</td>
<td>Ind Syn Ind</td>
<td>4.62 −4.00 4.49 3.18 3.22</td>
</tr>
<tr>
<td>100-GNT</td>
<td></td>
<td>D 64 32 32</td>
<td>Syn Ind NA</td>
<td>−4.15 3.00 NA −4.30 NA</td>
</tr>
<tr>
<td>178-GNA</td>
<td>C2 128 32 8</td>
<td>Ind Ind Ind</td>
<td>3.80 3.92 3.92 4.40 4.10</td>
<td></td>
</tr>
<tr>
<td>186-GNT</td>
<td>E 128 16 4</td>
<td>Ind Ind Syn</td>
<td>3.44 3.62 3.92 −5.00 3.40</td>
<td></td>
</tr>
<tr>
<td>U 154</td>
<td></td>
<td>C2 16 4 1</td>
<td>Syn# Ind Syn</td>
<td>−4.30# −4.85 −4.30 −3.60# −3.93#</td>
</tr>
<tr>
<td>Total n with synergy at 24 h</td>
<td>2/10 2/10 1/9 3/10 1/9</td>
<td>2/10 4/10 1/9 5/10 1/9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total n with bactericidal activity</td>
<td>2/10 4/10 1/9 5/10 1/9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*A negative sign denotes a reduction of inoculum compared with time 0; values in bold are consistent with bactericidal activity.
†The ertapenem and meropenem concentration in the combination was 10 mg l$^{-1}$, except when noted otherwise.
‡Imipenem concentration in the combination.
$§$XDR, only *in vitro* susceptible to fosfomycin (b 692161), or colistin (U 75) or tigecycline (NT 2).
$||$XDR, *in vitro* susceptible to colistin and fosfomycin (U 184), or tigecycline and trimethoprim/sulfamethoxazole (U 154, SP 153), or tigecycline and colistin (100-GNT), or tigecycline and fosfomycin (178-GNA).
¶MDR, *in vitro* susceptible to tigecycline, trimethoprim/sulfamethoxazole and fosfomycin (178-GNA), or colistin, tigecycline, trimethoprim/sulfamethoxazole and fosfomycin (V 5600).
#Meropenem concentration in the combination was 0.5×MIC.
In this study. *In vitro* synergistic activity was noted for 5 out of 29 (17.2%) ertapenem-containing combinations, 6 out of 29 (20.7%) meropenem-containing combinations and 7 out of 38 imipenem-containing combinations (18.4%) (Table 1). Interactions between the carbapenems were also evaluated with regard to the antibiotic concentration used. The antibiotic concentration was characterized as sub-inhibitory if it was lower than the respective MIC. The ertapenem MICs of the studied isolates ranged from 64 to 128 mg l\(^{-1}\) (Table 1), and so all 30 ertapenem-containing combinations comprised sub-inhibitory concentrations of the drug and resulted in the above-mentioned synergy of 17.2%. Twenty-nine meropenem-containing combinations comprised sub-inhibitory concentrations of meropenem and 6 (20.7%) resulted in synergy, whereas 20 of the imipenem-containing combinations comprised sub-inhibitory concentrations of this antibiotic and 3 (15.0%) resulted in synergy.

Seven (24.1%) ertapenem-, eight (27.6%) meropenem- and 11 (28.9%) imipenem-containing combinations were bactericidal. Ertapenem alone (10 mg l\(^{-1}\)) did not show cidal activity against any of the tested isolates (ertapenem MIC, 64–128 mg l\(^{-1}\)). Imipenem alone at 10 mg l\(^{-1}\) was bactericidal against two of the tested isolates (NT 2 and U 154), which exhibited the lowest imipenem MIC of 1–2 mg l\(^{-1}\).

The combination of imipenem (10 mg l\(^{-1}\))/ertapenem (10 mg l\(^{-1}\)) exhibited bactericidal activity against four of the tested isolates (imipenem MIC, 1–8 mg l\(^{-1}\) \(P=0.628\) versus imipenem and \(P=0.043\) versus ertapenem). Meropenem alone (10 mg l\(^{-1}\)) only exhibited cidal activity against one isolate (U 154), which exhibited the lowest meropenem MIC of 4 mg l\(^{-1}\), whereas imipenem (10 mg l\(^{-1}\))/meropenem (10 mg l\(^{-1}\)) was a cidal combination against five isolates (imipenem MIC, 1–32 and meropenem MIC, 4–32 mg l\(^{-1}\) \(P=0.076\) versus meropenem and \(P=0.350\) versus imipenem). The combination of meropenem (10 mg l\(^{-1}\)) with ertapenem (10 mg l\(^{-1}\)) was bactericidal against two isolates (meropenem MIC, 4–32 and ertapenem MIC, 16–64 mg l\(^{-1}\) \(P=0.237\) versus ertapenem and \(P=0.605\) versus meropenem). The cidal activity of all the above-mentioned combinations was apparent after 3 h of incubation and was sustained until the end of the 24 h experiment. Ertapenem/imipenem versus NT 2 and U 154 as well as meropenem/imipenem versus NT 2 were rapidly bactericidal after 1 h of incubation. The results of killing experiments are summarized in Fig. 1.

![Figure 1](https://www.microbiologyresearch.org/fig1.png)

**Fig. 1.** The difference between the mean starting inoculum and the mean viable cell count (\(Δ\log_{10}\) c.f.u. ml\(^{-1}\)) of the 10 *K. pneumoniae* isolates over time after (a) *in vitro* exposure to 10 mg l\(^{-1}\) concentrations of imipenem (IMP), ertapenem (ERT) or meropenem (MEM) alone and in dual combinations, representing the mean concentration of the free drug achievable in human serum after administration of the recommended dose, and (b) *in vitro* exposure to sub-inhibitory concentrations (0.5×MIC for isolates with an MIC \(≤8\) mg l\(^{-1}\) or 10 mg l\(^{-1}\) for isolates with an MIC \(≥16\) mg l\(^{-1}\)) of IMP, ERT and MEM alone and in dual combinations.
Table 2 depicts the overall beneficial effect of any combination compared to each antibiotic alone at different time points. Ertapenem/imipenem (10 mg l⁻¹⁻) achieved a mean reduction of viable counts from 4.92 to 2.98 log₁₀ c.f.u. ml⁻¹ from 3 until 24 h of exposure compared to ertapenem alone. The combination of meropenem (10 mg l⁻¹⁻)/imipenem (10 mg l⁻¹⁻) achieved a mean reduction of viable counts from 2.78 to 3.14 compared to meropenem alone from 3 until 24 h of exposure and a mean reduction of 2.46 log₁₀ c.f.u. ml⁻¹ at 24 h compared to imipenem alone. Importantly, the sub-inhibitory concentration-combinations, exhibited improved activity over each antibiotic alone up to at least 7 h of exposure.

This study showed that dual combinations of carbapenems were synergistic against MDR or XDR K. pneumoniae isolates harbouring blaoXA-48. Our results indicate that ertapenem-, imipenem- or meropenem-containing combinations even exhibit synergy and bactericidal activity at sub-inhibitory concentrations when evaluated against some of these isolates. When all OXA-48-producing isolates were examined together (Table 2), all of the tested combinations showed improved activity compared to single antibiotics, with the exception of ertapenem/meropenem (10 mg l⁻¹⁻).

This approach has not been previously studied against OXA-48 producers, except in an in vitro study conducted by Poirel et al. [18]. In that study, a variety of double-carbapenem combinations was tested against carbapenemase producers, including six OXA-48-producing K. pneumoniae clinical isolates. Using checkerboard, synergy was obtained for four out of six isolates, specifically with the imipenem-containing combinations. Time–kill assays only confirmed the significant synergy of ertapenem/imipenem and imipenem/meropenem combinations against the two OXA-48-producing strains. Interestingly, the same combinations did not show synergy when tested against two laboratory-derived OXA-48-producing Escherichia coli strains.

To date, reports on the activity of antimicrobials alone or in combinations against OXA-48 producers are limited. Third-generation cephalosporins remain active against ESBL-negative OXA-48-producing isolates [19, 20]. Unfortunately, ESBLs are frequently associated with OXA-48, limiting the use of these agents. Carbapenem monotherapy, on the other hand, has been proven to be an unreliable treatment option for infections by OXA-48 producers in both the animal model and the clinical setting [20, 21]. In our study, even for isolates with the lowest carbapenem MICs (U 154, NT 2), enhanced bactericidal activity and/or synergy was exhibited when they were exposed to a dual-carbapenem combination.

The preclinical evaluation of the recently approved combination of ceftazidime/avibactam, a non-β-lactam β-lactamase inhibitor that selectively inhibits class D OXA-48, rapid emergence of resistance has been reported [23].

Table 2. Mean difference between viable counts in the presence of the combination and viable counts in the presence of each antibiotic alone at different time points (Δlog₁₀ c.f.u. ml⁻¹⁻)

<table>
<thead>
<tr>
<th>Antibiotic combination vs each antibiotic alone at the clinically relevant concentration of 10 mg l⁻¹⁻</th>
<th>1 h</th>
<th>3 h</th>
<th>5 h</th>
<th>7 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERT/IMP vs ERT</td>
<td>−1.66</td>
<td>−4.92</td>
<td>−5.91</td>
<td>−5.56</td>
<td>−2.98</td>
</tr>
<tr>
<td>ERT/IMP vs IMP</td>
<td>−0.54</td>
<td>−0.80</td>
<td>−0.85</td>
<td>−1.10</td>
<td>−1.62</td>
</tr>
<tr>
<td>MEM/IMP vs MEM</td>
<td>−1.23</td>
<td>−2.78</td>
<td>−3.49</td>
<td>−3.05</td>
<td>−3.14</td>
</tr>
<tr>
<td>MEM/IMP vs IMP</td>
<td>−0.43</td>
<td>−0.50</td>
<td>−0.83</td>
<td>−1.23</td>
<td>−2.46</td>
</tr>
<tr>
<td>ERT/MEM vs ERT</td>
<td>−0.34</td>
<td>−2.5</td>
<td>−3.56</td>
<td>−4.29</td>
<td>−1.33</td>
</tr>
<tr>
<td>ERT/MEM vs MEM</td>
<td>−0.02</td>
<td>−0.66</td>
<td>−1.17</td>
<td>−1.65</td>
<td>−0.66</td>
</tr>
</tbody>
</table>

Antibiotic combination vs each antibiotic alone at sub-inhibitory concentration

<table>
<thead>
<tr>
<th>Antibiotic combination vs each antibiotic alone at sub-inhibitory concentration</th>
<th>1 h</th>
<th>3 h</th>
<th>5 h</th>
<th>7 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERT*/IMP† vs ERT*</td>
<td>−0.08</td>
<td>−2.02</td>
<td>−3.04</td>
<td>−2.69</td>
<td>−0.51</td>
</tr>
<tr>
<td>ERT*/IMP† vs IMP†</td>
<td>−0.15</td>
<td>−1.89</td>
<td>−2.42</td>
<td>−2.00</td>
<td>−0.84</td>
</tr>
<tr>
<td>MEM*/IMP† vs IMP†</td>
<td>−0.47</td>
<td>−3.18</td>
<td>−3.92</td>
<td>−3.71</td>
<td>−1.01</td>
</tr>
<tr>
<td>MEM*/IMP† vs MEM†</td>
<td>−0.42</td>
<td>−2.00</td>
<td>−2.51</td>
<td>−2.23</td>
<td>−0.44</td>
</tr>
<tr>
<td>ERT*/MEM† vs ERT*</td>
<td>−0.27</td>
<td>−2.48</td>
<td>−3.53</td>
<td>−4.29</td>
<td>−1.33</td>
</tr>
<tr>
<td>ERT*/MEM† vs MEM†</td>
<td>0</td>
<td>−0.7</td>
<td>−1.22</td>
<td>−1.93</td>
<td>−1.06</td>
</tr>
</tbody>
</table>

*ERT at sub-inhibitory concentration of 10 mg l⁻¹⁻.
†IMP at sub-inhibitory concentration of 0.5×MIC for all isolates except 100-GNT, for which 10 mg l⁻¹⁻ was used.
‡MER at sub-inhibitory concentration of 10 mg l⁻¹⁻ for all isolates except U 154, for which 0.5×MIC was used.
As the optimal treatment for infections by OXA-48 producers remains undefined, research on in vitro active combinations is ongoing. Along these lines, our study aimed to answer the question of whether combining two carbapenems would offer any benefit in vitro. We hypothesized that OXA-48 would act similarly to KPC, with both being serine β-lactamases. We expected that imipenem, due to its higher affinity with the OXA-48 enzyme [6], might act as a suicide inhibitor and be an advantageous component of double-combination regimens, as shown previously for KPC-producing *Klebsiella pneumoniae* [24–27]. Synergy and bactericidal activity were observed for all possible double-carbapenem combinations tested at ranges of 17.2–20.7 % and 24.1–28.9 %, respectively. The imipenem-containing combinations showed particularly enhanced in vitro activity, even in sub-inhibitory concentrations, compared to single agents and no antagonism was observed with any of the isolate-combinations tested.

The benefit of the combinations tested in this study was shown to be strain-specific, as it is probable that other genetic or phenotypic characteristics of the isolates, besides the presence of β-lactamase-encoding genes, play an important role in the expression of synergy. This is consistent with most of the in vitro synergy studies published to date and highlights the need for routine implementation of prior in vitro synergy testing to guide individualized treatment decisions for difficult-to-treat infections caused by MDR, XDR or PDR Gram-negative bacteria.

In summary, this report expands current knowledge regarding the efficacy of double-carbapenem regimens against OXA-48-producing *K. pneumoniae* and suggests that improved in vitro activity can be offered against some of these isolates, including those with high carbapenem MICs. A clinical evaluation of this therapeutic approach will define its role in the management of infections by the increasingly prevalent OXA-48-producing *Enterobacteriaceae*.

**References**


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