**In vitro evaluation of antibiotic synergy for NDM-1-producing Enterobacteriaceae**

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**Objectives:** To analyse the in vitro activity of colistin, fosfomycin and tigecycline alone or in combination against enterobacterial NDM-1 producers.

**Methods:** MIC values of colistin, fosfomycin and tigecycline were determined for 28 NDM-1-producing enterobacterial isolates. In vitro synergy combination testing was performed for eight clinical isolates and one *Escherichia coli* transconjugant (six being susceptible to the three antibiotics) using microdilution and checkerboard techniques.

**Results:** MICs of colistin, fosfomycin and tigecycline were determined, showing that one-third of NDM-1-producing isolates were resistant or intermediate to at least one of the three drugs. nevertheless, in vitro synergistic activity was observed for colistin plus fosfomycin and colistin plus tigecycline in very rare cases.

**Conclusions:** Synergistic activity was observed for colistin and fosfomycin, and colistin and tigecycline in rare cases, most of the interactions being indifferent.

**Keywords:** carbapenemases, checkerboard technique, colistin, fosfomycin, tigecycline

**Introduction**

Carbapenem-hydrolysing β-lactamases such as KPC type (Ambler class A), IMP and VIM types (class B) and OXA-48 (class D) are now reported worldwide among Enterobacteriaceae. Recently, the class B carbapenemase NDM-1 (New Delhi metallo-β-lactamase) has been identified mostly from Enterobacteriaceae, mainly from India, Pakistan, Bangladesh and the UK, but also from many countries worldwide.1–3 Most of the NDM-1 producers are resistant to all β-lactams including carbapenems, to all aminoglycosides and to fluoroquinolones, nitrofurantoin and sulfonamides and are susceptible only to two bactericidal antibiotics (fosfomycin and colistin) and one bacteriostatic antibiotic (tigecycline).2 Therapies using colistin plus fosfomycin, colistin plus tigecycline or fosfomycin plus tigecycline are not commonly considered in medical practice. The objective of this study was to analyse the in vitro activity of colistin, fosfomycin and tigecycline alone or in combination against NDM-1 producers.

**Materials and methods**

The susceptibility of 28 isolates of NDM-1 producers, comprising 10 *Escherichia coli*, 10 *Klebsiella pneumoniae*, 4 *Enterobacter cloacae*, 1 *Klebsiella oxytoca*, 1 *Providencia rettgeri*, 1 *Citrobacter freundii* and 1 *E. coli* J53 transconjugant (previously obtained in our laboratory from an *E. coli* isolate as donor),2,3 was determined for fosfomycin, colistin and tigecycline. These isolates were of worldwide origin (India, France, Kenya, Sultanate of Oman and Australia).2 The MICs of colistin, fosfomycin, tigecycline and their combinations were determined by using the broth microdilution technique as recommended by the CLSI guidelines.4,5 The *E. coli* J53 isolate, susceptible to all antibiotics, was used for quality control. Checkerboard synergy testing was performed in duplicate with three resistant isolates and six susceptible isolates, including the *E. coli* J53 transconjugant expressing NDM-1 as previously described. The fractional inhibitory concentration (FIC) was calculated according to the formula \( \sum \text{FIC} = \text{FIC of drug A} + \text{FIC of drug B} \), where FIC of drug A or B = MIC of drug A or B in combination divided by the MIC of drug A or B alone. Interpretation of the results was based on the following: \( \sum \text{FIC} \) values of \( \leq 0.5 \) indicate synergism; \( \sum \text{FIC} \) values of \( >0.5 \) to 4 indicate no interaction; and \( \sum \text{FIC} \) values of \( >4 \) indicate antagonism.6

**Results and discussion**

NDM-1 producers collected worldwide are all multidrug resistant but many remain susceptible to fosfomycin, colistin and tigecycline.2 For the 28 NDM-1 producers tested in this study, 33% were resistant or intermediate to at least one of those drugs.
antibiotics (Figure 1). Two isolates were resistant to colistin (one *K. pneumoniae* and one *P. rettgeri*), four isolates were resistant to fosfomycin (three *K. pneumoniae* and one *E. cloacae*) and seven isolates exhibited high MICs of tigecycline (five *K. pneumoniae*, one *C. freundii* and one *P. rettgeri*). All the *E. coli* isolates and 4 out of the 10 *K. pneumoniae* isolates were susceptible to the three drugs.

For the chequerboard synergy testing, the results of the present study show that combinations of these drugs in pairs result mainly in no interaction (Table 1). In addition, no antagonism (defined by an FIC index >4) was noted with any of the three combinations tested. Interestingly, *in vitro* synergistic activity against NDM-1-producing isolates was noted with the combinations of colistin plus fosfomycin and colistin plus tigecycline, in rare cases for susceptible isolates. These latter results may be consistent with previous time–kill studies showing that the combination of colistin plus tigecycline was synergistic for the treatment of infections caused by a multiresistant VIM-1 and SHV-12 β-lactamase-producing *K. pneumoniae* epidemic strain. Two other studies demonstrated *in vitro* synergy between colistin or polymyxin B, another member of the class of polymyxins, and tigecycline against KPC-producing Enterobacteriaceae. A recent time–kill study reported that fosfomycin resulted in synergy with colistin against 11.8% of KPC-2-producing *K. pneumoniae* isolates.

Considering clinical infections due to KPC-producing Enterobacteriaceae, an analysis of different studies reported that 66% of patients treated with polymyxin plus tigecycline combinations

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**Figure 1.** MIC distributions of fosfomycin, colistin and tigecycline as determined by a broth microdilution technique. The CLSI breakpoints for colistin (CST), fosfomycin (FOF) and tigecycline (TGC), categorizing isolates as ‘susceptible’, ‘intermediate’ or ‘resistant’, are indicated by arrows.

**Table 1.** FIC variations and chequerboard synergy testing for nine NDM-1 producers

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Country of isolation</th>
<th>MIC (mg/L) FIC variation</th>
<th>Chequerboard synergy testing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CST</td>
<td>FOF</td>
</tr>
<tr>
<td><strong>Susceptible isolates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli A</em></td>
<td>Australia</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td><em>E. cloacae D</em></td>
<td>India</td>
<td>0.5</td>
<td>8</td>
</tr>
<tr>
<td><em>K. pneumoniae A</em></td>
<td>Kenya</td>
<td>0.5</td>
<td>64</td>
</tr>
<tr>
<td><em>K. pneumoniae C</em></td>
<td>Sultanate of Oman</td>
<td>0.5</td>
<td>32</td>
</tr>
<tr>
<td><em>K. oxytoca A</em></td>
<td>India</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td><em>E. coli J53 transconjugant</em> (with <em>E. coli A</em> as donor)</td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Resistant isolates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. cloacae A</em></td>
<td>India</td>
<td>1</td>
<td>256</td>
</tr>
<tr>
<td><em>K. pneumoniae E</em></td>
<td>India</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td><em>P. rettgeri</em></td>
<td>India</td>
<td>256</td>
<td>32</td>
</tr>
</tbody>
</table>

TGC, tigecycline; CST, colistin; FOF, fosfomycin; NI, no interaction.

Results represent interpretations of the minimum and maximum FIC values.

aResistant to at least one antibiotic.
and 71% of patients treated with tigecycline monotherapy were cured. In contrast, only 40% and 14% of patients were treated successfully by carbapenem and polymyxin monotherapy, respectively.11 In addition, when polymyxin B was used in monotherapy, decreased susceptibility to this drug was observed for 25% of the isolates.12 Other studies reported that the colistin plus tigecycline combination allowed cure of persistent bacteraemia due to carbapenem-resistant K. pneumoniae,13 but a monotherapy regimen using tigecycline was also reported to cure mediastinitis due to a VIM-1-producing K. pneumoniae.14 A recent study indicated that the tigecycline and colistin combination was bactericidal against KPC-producing isolates in comparison with a monotherapy regimen using tigecycline and might be a therapeutic option for infections due to multidrug-resistant KPC producers when some bactericidal activity is necessary, such as in bacteraemia, endocarditis or other severe infections.9

The results of the present study deserve additional in vitro and in vivo investigations. Although synergy between tigecycline, fosfomycin and colistin was rarely observed here, at least no antagonism was observed.

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Transparency declarations
None to declare.

References