Infections with carbapenemase-producing *Klebsiella pneumoniae* (KPC) are emerging causes of morbidity and mortality at institutions worldwide (2, 11, 14–16). KPC isolates are defined by resistance to carbapenems and are generally resistant to most other antimicrobial classes. Salvage agents that may retain activity against KPC isolates include colistin, gentamicin, doxycycline, and tigecycline (8, 9, 18). Resistance to these agents is increasing (1, 6), however, and treatment failures are well recognized with each (8). Given the dearth of effective antimicrobial agents and extensively drug-resistant (XDR) isolates from our encouraging preliminary experience in treating infections with carbapenemase-producing *Klebsiella pneumoniae* (KPC) isolates by time-kill. The combination of doripenem and colistin reduced the starting inocula by 2 logs for each isolate (range, 2.02 to 6.01 log_{10}) and was bactericidal and synergistic against 75 and 50%, respectively. Among colistin- and pan-drug-resistant isolates, synergy was identified in 60 and 67%, respectively. All other combinations were inferior. We are currently evaluating the combination of doripenem and colistin as a frontline therapy for KPC infection.

We tested two-drug combinations of doripenem, colistin, gentamicin, and doxycycline against 12 carbapenemase-producing *Klebsiella pneumoniae* (KPC) isolates by time-kill. The combination of doripenem and colistin reduced the starting inocula by 2 logs for each isolate (range, 2.02 to 6.01 log_{10}) and was bactericidal and synergistic against 75 and 50%, respectively. Among colistin- and pan-drug-resistant isolates, synergy was identified in 60 and 67%, respectively. All other combinations were inferior. We are currently evaluating the combination of doripenem and colistin as a frontline therapy for KPC infection.

**TABLE 1** MICs for KPC isolates*.

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (µg/ml)</th>
<th>Log Δ from the starting inoculum (log_{10} CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Doripenem</td>
<td>Colistin</td>
</tr>
<tr>
<td>1*</td>
<td>64</td>
<td>8</td>
</tr>
<tr>
<td>18</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>82</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>124*</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>133*</td>
<td>128</td>
<td>16</td>
</tr>
<tr>
<td>136</td>
<td>&gt;128</td>
<td>4</td>
</tr>
<tr>
<td>141</td>
<td>&gt;128</td>
<td>0.5</td>
</tr>
<tr>
<td>145*</td>
<td>&gt;128</td>
<td>16</td>
</tr>
<tr>
<td>167</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td>180*</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>182*</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>183</td>
<td>64</td>
<td>16</td>
</tr>
</tbody>
</table>

*The following susceptibility breakpoints were used: doripenem and colistin, ≤2 µg/ml, and gentamicin and doxycycline, ≤4 µg/ml.

Log Δ = Final inoculum − starting inoculum (log_{10} CFU/ml). Negative numbers represent a decrease from the starting inoculum, and positive numbers represent an increase.

Fixed concentrations of doripenem (8 µg/ml), colistin (1 µg/ml), gentamicin (2 µg/ml), and doxycycline (2 µg/ml) were used for all isolates.

*Indicates isolates resistant to all agents tested.
Synergy and antagonism were defined as concentrations that were representative of achievable serum levels. Antimicrobial assays (12), we found that colistin was bactericidal (1), killing 2-log10 greater and 1.61 log10 decrease from the starting inoculum against 33% (4/12) isolates and 50% (6/12) of isolates, respectively. Doripenem plus doxycycline was synergistic against 25% (3/12) of isolates, respectively. Synergy was achieved against isolates that were doxycycline susceptible; bactericidal activity or synergy was not identified among doxycycline-resistant strains. Doripenem plus gentamicin was also bactericidal against 25% (3/12), but synergy was evident against only 8% (1/12). The least active combination was colistin plus doxycycline and doripenem plus doxycycline, which were bactericidal against 17% (2/12) and 8% (1/12) of isolates, respectively. Doripenem plus doxycycline was synergistic against 25% (3/12) of isolates, whereas colistin plus doxycycline was synergistic against 8% (1/12). Antagonism was identified with all combinations except doripenem plus colistin (Table 3). The combination of colistin and doxycycline was antagonistic in 25% (3/12).

Doripenem plus colistin and doripenem plus gentamicin were associated with the lowest and highest median areas under the curve (AUCs), respectively (Table 4). Indeed, the median AUC for colistin plus doripenem was lower than those for the other combinations (P = 0.004), including the individual combinations of gentamicin plus doxycycline (P = 0.04) and doripenem plus doxycycline (P < 0.0001) (Fig. 1) (GraphPad Prism Software, La Jolla, CA).

Taken together, our data demonstrate that doripenem plus colistin was the most active two-drug combination against KPC isolates. Doripenem plus colistin attained the highest levels of bactericidal activity and synergy, and it was the only combination that did not show any antagonism. Moreover, the combination was effective at clinically achievable concentrations of both drugs (3, 7) and it was particularly active against colistin- and pan-drug-resistant isolates. In this regard, our findings are similar to those we reported against XDR Acinetobacter baumannii isolates from our center (17). Furthermore, our results extend our understanding of in vitro synergy against KPC isolates, which have been studied in colistin-susceptible strains (10, 20, 22) or by checkerboard broth microdilution (5) previously.

At present, the treatment options for patients with KPC infections are limited, as evident by crude mortality rates that are reported to range from 42 to 69% (13, 14, 19, 24). Mortality rates are

### TABLE 2 Log change (log10 CFU/ml) from the starting inoculum and most active single agent after 24 h of incubation

<table>
<thead>
<tr>
<th>Combination</th>
<th>Log Δ (log10 CFU/ml) for isolate:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Colistin + doripenem</td>
<td>6.04</td>
</tr>
<tr>
<td>Colistin + gentamicin</td>
<td>4.56</td>
</tr>
<tr>
<td>Colistin + doxycycline</td>
<td>2.75</td>
</tr>
<tr>
<td>Doripenem + gentamicin</td>
<td>3.69</td>
</tr>
<tr>
<td>Doripenem + doxycycline</td>
<td>2.47</td>
</tr>
<tr>
<td>Gentamicin + doxycycline</td>
<td>5.87</td>
</tr>
</tbody>
</table>

### TABLE 3 Interactions between two drugs tested in combination

<table>
<thead>
<tr>
<th>Backbone agent</th>
<th>Agent in combination</th>
<th>Synergy, Δn (%)</th>
<th>Indifference, Δn (%)</th>
<th>Antagonism, Δn (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colistin</td>
<td>Doripenem</td>
<td>6 (50)</td>
<td>6 (50)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Gentamicin</td>
<td>3 (25)</td>
<td>8 (67)</td>
<td>1 (8)</td>
</tr>
<tr>
<td></td>
<td>Doxycycline</td>
<td>1 (8)</td>
<td>8 (67)</td>
<td>3 (25)</td>
</tr>
<tr>
<td>Doripenem</td>
<td>Gentamicin</td>
<td>1 (8)</td>
<td>9 (75)</td>
<td>2 (17)</td>
</tr>
<tr>
<td></td>
<td>Doxycycline</td>
<td>3 (25)</td>
<td>7 (58)</td>
<td>2 (17)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>Doxycycline</td>
<td>5 (42)</td>
<td>5 (42)</td>
<td>2 (17)</td>
</tr>
</tbody>
</table>

*Log Δ is defined as follows. Nonshaded rows: final inoculum = starting inoculum (log10 CFU/ml). Negative numbers represent a decrease from the starting inoculum, and positive numbers represent an increase. Shaded rows: final inoculum of the most active single drug = final inoculum of the two-drug combination (log10 CFU/ml). Negative numbers represent greater killing with two-drug combinations versus a single drug. Fixed concentrations of doripenem (8 μg/ml), colistin (1 μg/ml), gentamicin (2 μg/ml), and doxycycline (2 μg/ml) were used for all isolates. Bold numbers indicate synergy.*
higher among patients infected with colistin-resistant isolates (24). Investigators have reported that combination therapy may improve outcomes of KPC infections compared to those achieved with monotherapy (8, 23), but optimal regimens are not defined and none have been shown to be effective against colistin-resistant isolates. Of note, we reported promising preliminary data in a pilot clinical study using doripenem plus colistin to treat XDR \textit{A. baumannii} infections (17). Based on these data and the \textit{in vitro} findings of the current study, we are currently using the combination as the recommended front-line regimen against KPC infections.

The other combinations tested in this study demonstrated inferior bactericidal activity and less synergy. Furthermore, the second most potent combination \textit{in vitro} (colistin plus gentamicin) is limited in clinical settings by the potential for additive toxicity. Our data suggest that doxycycline plus gentamicin may be useful against isolates that retain susceptibility to the former agent, but at our center such isolates are in the minority. In the end, this combination was synergistic against only 25% (3/12) of isolates and may be unsuitable for the treatment of severe or life-threatening KPC infections. At present, we reserve doxycycline-containing regimens for the treatment of uncomplicated cystitis at our center. The remaining combinations were even less effective \textit{in vitro}, and we do not employ them in the clinic. Finally, it is important to note that the number of strains evaluated here is limited and future studies are needed. Toward this, the exact mechanisms underlying the synergistic effects between doripenem and colistin have not been identified and merit further investigation. It is possible that colistin permeabilizes the outer cell membrane of KPC isolates, allowing high concentrations of doripenem to access penicillin-binding proteins in the cytoplasmic membrane where the drug acts.

\begin{table}[h]
\centering
\caption{AUCs for two-drug combinations after 24 h of incubation}
\begin{tabular}{llllll}
\hline
\textbf{Isolate} & \textbf{Colistin} & \textbf{Doripenem} & \textbf{Gentamicin plus doxycycline} & \textbf{Gentamicin plus doxycycline} \\
& \textbf{Plus doripenem} & \textbf{Plus gentamicin} & \textbf{Plus doxycycline} & \textbf{Plus doripenem} & \textbf{Plus gentamicin} & \textbf{Plus doxycycline} \\
\hline
1 & 62.34 & 88.02 & NA & 209.5 & NA & 106.6 \\
18 & 69.6 & 103.9 & NA & 81.7 & NA & 134.2 \\
82 & 74.8 & 57.4 & 141.0 & 62.8 & 150.6 & 52.9 \\
124 & 47.8 & 37.46 & 132.5 & NA & 128.2 & NA \\
133 & 87.9 & 110.2 & NA & 176.5 & 168.5 & 206.0 \\
136 & 84.8 & 121.4 & 138.5 & 202.9 & 213.6 & 143.4 \\
141 & 59.4 & 29.1 & 99.9 & 22.4 & 156.7 & 50.8 \\
145 & 81.5 & 139.0 & 157.4 & NA & 195.3 & NA \\
167 & 33.9 & NA & 51.0 & 207.6 & 207.4 & 220.1 \\
180 & 118.3 & 132.4 & 146.7 & 155.4 & 138.7 & 203.7 \\
182 & 112.3 & 137.0 & 135.5 & 183.8 & 164.8 & 168.2 \\
183 & 82.1 & 10.0 & 132.1 & 15.9 & 107.5 & 26.4 \\
Median (range) & 78.2 (33.9–118.3) & 103.9 (10.0–139.0) & 135.5 (51.0–157.4) & 165.9 (15.9–209.5) & 160.8 (107.5–213.6) & 138.8 (26.4–220.1) \\
\hline
\end{tabular}
\begin{flushleft}
\textsuperscript{a}NA, not applicable; area under the curve was not calculated for antagonistic combinations.
\end{flushleft}
\end{table}

\begin{figure}[h]
\centering
\begin{subfigure}{0.3\textwidth}
\centering
\includegraphics[width=\textwidth]{a}
\caption{Representative area under the curve (AUC) for isolates alone and in combination for the following drug combinations: (a) colistin (dark gray) plus doripenem (light gray); (b) gentamicin (light gray) plus doxycycline (dark gray); and (c) doripenem (light gray) plus doxycycline (dark gray). Black shading represents the AUC for two-drug combinations.}
\end{subfigure}
\end{figure}
In conclusion, it is important to stress that these results were obtained against KPC isolates from a single center. As such, the results may not be applicable to isolates from other centers or geographic locations. It is advisable for centers to test isolates recovered from patients at their institution. Having introduced doripenem plus colistin as the recommended therapy for KPC infections at our center, we are in the process of accumulating our clinical experience and assessing the impact of the regimen on preventing the emergence of colistin resistance.

ACKNOWLEDGMENTS

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