Activity of a novel aminoglycoside, ACHN-490, against clinical isolates of Escherichia coli and Klebsiella pneumoniae from New York City

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Objectives: Reports of Enterobacteriaceae resistant to all commonly used antimicrobial agents, including β-lactams, fluoroquinolones and aminoglycosides, are increasing in hospitals worldwide. The activity of ACHN-490, a next-generation aminoglycoside, was examined against clinical isolates of Escherichia coli and Klebsiella pneumoniae from hospitals in New York City, an area where multidrug-resistant organisms are endemic.

Methods: Unique patient isolates of E. coli and K. pneumoniae were gathered from 16 hospitals located in New York City in 2009 and underwent susceptibility testing to aminoglycosides and ACHN-490. Subsets of isolates were characterized by PCR for the presence of genes encoding aminoglycoside-modifying enzymes, ribosomal methylases and KPC-type carbapenemases.

Results: Although most isolates of E. coli were susceptible to the aminoglycosides, the MIC90 values of gentamicin, tobramycin and amikacin were 32, 8 and 4 mg/L, respectively. The MIC90 of ACHN-490 was 1 mg/L. Multidrug resistance, including resistance to aminoglycosides and the presence of blaKPC, was much more common in isolates of K. pneumoniae. However, the MIC90 of ACHN-490 for K. pneumoniae was also 1 mg/L. The MICs of ACHN-490 did not correlate with the presence of commonly recovered aminoglycoside-modifying enzymes. Bactericidal activity was evident in most isolates at concentrations 4× the MIC.

Conclusions: The novel aminoglycoside ACHN-490 retains activity against most isolates of E. coli and K. pneumoniae, including multidrug-resistant strains. Additional studies examining the roles of efflux systems and outer membrane permeability alterations are recommended in isolates with reduced susceptibility to this agent.

Keywords: antimicrobial resistance surveillance, multidrug resistant, mechanisms of resistance

Introduction

The emergence and rapid spread of multidrug-resistant Gram-negative pathogens in hospitals throughout the world has been alarming. The spread of Klebsiella pneumoniae possessing the carbapenemase KPC has been especially disconcerting. Originally confined to isolates in the north-eastern USA, KPC-producing isolates have now become commonplace in New York City and have been reported throughout North America and in Asia, South America and Europe.1,2 In addition, isolates of Escherichia coli carrying the KPC β-lactamase have also been identified in New York City.3 Already resistant to β-lactam agents, many of the KPC-producing isolates are also resistant to fluoroquinolones and older aminoglycosides (for example gentamicin, tobramycin and amikacin), leaving only extremely limited therapeutic options. The urgent need for new antimicrobial agents has been highlighted by the recent “10x’20 initiative” by the Infectious Diseases Society of America.4

ACHN-490 is a derivative of sisomicin with enhanced activity against many multidrug-resistant Gram-negative and Gram-positive bacteria.5–8 Preliminary studies indicate bactericidal activity against several Gram-negative pathogens.9 In this report, we determined the activity of ACHN-490 against contemporary clinical isolates of E. coli and K. pneumoniae from hospitals in New York City.

Materials and methods

Bacterial isolates

Single-patient isolates of E. coli and K. pneumoniae were gathered from hospital microbiology laboratories during a 3 month surveillance study...
conducted in 2009, as previously described.\textsuperscript{1} Participating hospitals included the 15 hospitals in Brooklyn, NY, and one hospital in Staten Island, NY. Susceptibility testing was performed on all isolates using the agar dilution method, according to established guidelines.\textsuperscript{10} ACHN-490 was kindly supplied by Achaogen, Inc. (South San Francisco, CA, USA). Based on their various aminoglycoside MICs, a subset of isolates was selected for additional studies to investigate the potential mechanisms of resistance. Time–kill studies were performed on some of these isolates using 4× the MIC of ACHN-490 (MIC range 0.25–4 mg/L).\textsuperscript{11} Bactericidal activity was defined as a ≥3 log drop in the inoculum after 24 h of incubation.\textsuperscript{11} For some isolates, MICs were repeated by the broth microdilution method with and without the presence of phenyl-arginine–β-naphthylamide (PABN) and 32 mg/L) to all three aminoglycosides were screened \(×\) the MIC of ACHN-490 (MIC range 0.25–4 mg/L) to all three aminoglycosides were screened \(×\) the MIC of ACHN-490. PABN was included at 100 mg/L, representing 0.5\(×\) the MIC for the tested isolates. Results

E. coli

A total of 3050 isolates of \(E.\ \text{coli}\) were collected during the surveillance study involving the 16 hospitals (Table 1). Although most were susceptible to the three aminoglycosides, the MIC\(_{90}\) values for gentamicin, tobramycin and amikacin were 32, 8 and 4 mg/L, respectively. Only three isolates (0.1\%) had MICs of ACHN-490 ≥8 mg/L; two were obtained from urine cultures and one was from a blood culture. A subset of 36 isolates, including one with \(\text{bla}_{\text{KPC}}\), was selected based on the MICs of gentamicin, tobramycin, amikacin and ACHN-490 (Table 2). Among these isolates, 61\% were resistant to ciprofloxacin and 44\% to trimethoprim/sulfamethoxazole, and 56\% had a ceftazi-dime MIC >1 mg/L. Isolates were grouped according to the aminoglycoside-modifying enzymes genes that were detected.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{MIC} (mg/L) & \textbf{MIC\(_{50}\)} & \textbf{MIC\(_{90}\)} & \textbf{range} & \textbf{Susceptible} & \textbf{Intermediate} & \textbf{Resistant} \\
\hline
\textbf{Gentamicin} & 0.5 & 32 & ≤0.25 to >64 & 86.5\% & 0.6\% & 12.9\% \\
\textbf{Tobramycin} & 0.5 & 8 & ≤0.12 to >64 & 86.9\% & 6.2\% & 6.9\% \\
\textbf{Amikacin} & 2 & 4 & ≤0.5 to >64 & 99.1\% & 0.4\% & 0.5\% \\
\textbf{ACHN-490\textsuperscript{a}} & 0.5 & 1 & ≤0.06 to >8 & & & \\
\hline
\end{tabular}
\caption{Susceptibility data for 3050 isolates of \(E.\ \text{coli}\) gathered in 2009}
\end{table}

\textsuperscript{a}Clinical breakpoints have not been defined.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{No. of isolates} & \textbf{Genes detected} & \textbf{Predicted resistance} & \textbf{GEN} & \textbf{TOB} & \textbf{AMK} & \textbf{ACHN-490} \\
\hline
3 & \textbf{aac(3)-IIa; aadA5} & GEN, TOB, SIS & >32 & 8 to >32 & 2–4 & 0.5 to 1 \\
1 & \textbf{aac(6\textsuperscript{′})-Ib; aadA5} & TOB, AMK, SIS & 4 & >32 & >32 & 1 \\
10 & \textbf{aac(6\textsuperscript{′})-Ib} & TOB, AMK, SIS & 0.5 to >32 & 32 to >32 & 16 to >32 & 0.25 to >8 \\
1 & \textbf{aac(3)-IIa; aac(6\textsuperscript{′})-Ib} & GEN, TOB, AMK, SIS & >32 & >32 & 8 & 2 \\
1 & \textbf{aac(3)-IIa} & GEN, TOB, AMK, SIS & 32 & 8 & 4 & 4 \\
1 & \textbf{aac(6\textsuperscript{′})-Ib; ant(3\textsuperscript{′})-I} & TOB, AMK, SIS & >32 & >32 & >32 & 4 \\
19 & none & & <0.25 to >32 & 0.5 to >32 & 1 to >32 & 0.25 to 8 \\
\hline
\end{tabular}
\caption{Susceptibility profiles and the presence of aminoglycoside-modifying enzymes in 36 selected isolates of \(E.\ \text{coli}\)}
\end{table}

GEN, gentamicin; TOB, tobramycin; AMK, amikacin; SIS, sisomicin.
\textsuperscript{a}Time–kill studies were performed at 4× the MIC of ACHN-490.
Occasional isolates had aminoglycoside resistance, particularly to gentamicin, that could not be explained based on the screening for modifying enzymes. None of the isolates was found to carry ribosomal methylases. There was no clear correlation between the MICs of ACHN-490 and the presence of aminoglycoside-modifying enzymes. Of the three isolates that had ACHN-490 MICs of ≥8 mg/L, two possessed \( \text{aac(6\')-Ib} \) and one did not have genes for modifying enzymes detected. The \( \text{aac(6\')-Ib} \) gene was detected in 11 other isolates with lower ACHN-490 MICs. For the three highly ACHN-490-resistant isolates, only one had a 4-fold MIC reduction with the addition of PABN. Among these three isolates, two [both with \( \text{aac(6\')-Ib} \)] were resistant to tobramycin and amikacin, and one was resistant to gentamicin.

Time–kill studies were conducted with nine isolates with concentrations of ACHN-490 at 4× MIC (Table 2). Bactericidal killing was evident in seven isolates. Two isolates, possessing \( \text{aac(3)-IIa} \) and an integron-associated \( \text{aadA5} \), had evidence of regrowth at 24 h. Insertion of the integron carrying \( \text{aadA5} \) into a susceptible TOP10 *E. coli* did not change the MIC of ACHN-490 (0.25 mg/L) when compared with a TOP10 isolate without this enzyme. For the two isolates exhibiting regrowth, the isolates recovered after 24 h of incubation had an 8- to 16-fold increase in the MIC of ACHN-490. The addition of the efflux inhibitor NMP had no effect on the ACHN-490 MICs, and the addition of PABN caused a 4-fold decrease in one isolate. Only one of the two isolates exhibiting regrowth had a 4-fold rise in the MICs of gentamicin, tobramycin and amikacin.

### K. pneumoniae

During the surveillance study, 1155 isolates of *K. pneumoniae* were gathered. Although resistance to the aminoglycosides was common (Table 3), only two isolates (0.2%) had ACHN-490 MICs >8 mg/L. It is noteworthy that, although the two isolates originated from two separate hospitals, they belonged to the same ribotype (data not shown). Among the surveillance isolates with \( \text{bla}_{\text{OEC}} \), the ACHN-490 MIC\(_{50}\) and MIC\(_{90}\) were unchanged at 0.5 and 1 mg/L, respectively. Forty isolates (including 25 multidrug-resistant isolates with \( \text{bla}_{\text{OEC}} \)) were chosen for further analysis based on their range of MICs of the aminoglycosides (Table 4). Among these isolates, 80% were resistant to ciprofloxacin and 88% to trimethoprim/sulfamethoxazole, and 83% possessed extended-spectrum β-lactamases. As with the

#### Table 3. Susceptibility data for 1155 isolates of *K. pneumoniae* gathered in 2009

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>( \text{MIC}_{50} )</th>
<th>( \text{MIC}_{90} )</th>
<th>range</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>1</td>
<td>64</td>
<td>≤0.25 to &gt;64</td>
<td>71.2%</td>
<td>3.1%</td>
<td>25.7%</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>1</td>
<td>&gt;64</td>
<td>≤0.12 to &gt;64</td>
<td>52.5%</td>
<td>1.5%</td>
<td>46.0%</td>
</tr>
<tr>
<td>Amikacin</td>
<td>1</td>
<td>32</td>
<td>≤0.5 to &gt;64</td>
<td>70.4%</td>
<td>25.1%</td>
<td>4.5%</td>
</tr>
<tr>
<td>ACHN-490(^a)</td>
<td>0.5</td>
<td>1</td>
<td>0.12 to &gt;8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Clinical breakpoints have not been defined.

#### Table 4. Susceptibility profiles and presence of aminoglycoside-modifying enzymes in 40 selected isolates of *K. pneumoniae*

<table>
<thead>
<tr>
<th>No. of isolates</th>
<th>Genes detected</th>
<th>Predicted resistance</th>
<th>MIC (mg/L)</th>
<th>≥3 log kill in time–kill(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>( \text{aac(6')-Ib; ant(3')-I; ant(3')-Ia} )</td>
<td>TOB, AMK, SIS</td>
<td>8 to &gt;32</td>
<td>1/1</td>
</tr>
<tr>
<td>14</td>
<td>( \text{aac(6')-Ib; ant(3')-I} )</td>
<td>TOB, AMK, SIS</td>
<td>4 to &gt;32</td>
<td>2/2</td>
</tr>
<tr>
<td>4</td>
<td>( \text{aac(6')-Ib} )</td>
<td>TOB, AMK, SIS</td>
<td>4 to &gt;32</td>
<td>2/2</td>
</tr>
<tr>
<td>2</td>
<td>( \text{ant(3')-I} )</td>
<td>&lt;0.25–0.5</td>
<td>0.5</td>
<td>1/1</td>
</tr>
<tr>
<td>2</td>
<td>( \text{aac(3)-Ia; aac(6')-Ib} )</td>
<td>GEN, TOB, AMK, SIS</td>
<td>&gt;32</td>
<td>2/2</td>
</tr>
<tr>
<td>1</td>
<td>( \text{aac(6')-Ib; ant(3')-I; aph(3')1a} )</td>
<td>GEN, TOB, AMK, SIS</td>
<td>&gt;32</td>
<td>1/1</td>
</tr>
<tr>
<td>1</td>
<td>( \text{aac(6')-Ib; ant(3')-I; ant(3')-Ia; aph(3')1a} )</td>
<td>GEN, TOB, AMK, SIS</td>
<td>&gt;32</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>( \text{ant(3')-Ia} )</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>0.5–1</td>
</tr>
<tr>
<td>1</td>
<td>( \text{aac(6')-Ib; ant(2')-Ia; ant(3')-I} )</td>
<td>GEN, TOB, AMK, SIS</td>
<td>8</td>
<td>1/1</td>
</tr>
<tr>
<td>1</td>
<td>( \text{aac(6')-Ib; ant(3')-I; ant(2')-Ia; aac(6')-33} )</td>
<td>GEN, TOB, AMK, SIS</td>
<td>16</td>
<td>1/1</td>
</tr>
<tr>
<td>1</td>
<td>( \text{aac(6')-Ib; ant(2')-Ia} )</td>
<td>GEN, TOB, AMK, SIS</td>
<td>32</td>
<td>1/1</td>
</tr>
<tr>
<td>8</td>
<td>none</td>
<td>&lt;0.25 to &gt;32</td>
<td>0.5 to &gt;32</td>
<td>0.5–4</td>
</tr>
</tbody>
</table>

\(^a\)Time–kill studies were performed at 4× the MIC of ACHN-490.
E. coli isolates, gentamicin resistance could not be accounted for in several isolates, and genes for ribosomal methylases were not detected in any of the isolates. There was again no clear correlation between the presence of genes encoding aminoglycoside-modifying enzymes and the MICs of ACHN-490. The two isolates with high-level resistance to ACHN-490 had aac(6’)-Ib alone; this gene was also present in 27 isolates with lower MICs of ACHN-490. The two resistant isolates were also highly resistant to gentamicin, tobramycin and amikacin. For these two isolates, the addition of PABN had no appreciable effect on the ACHN-490 MICs. Fifteen isolates underwent time–kill studies, and in all 15 cases ACHN-490 had bactericidal activity.

Discussion
The emergence of multidrug-resistant Gram-negative nosocomial pathogens has created serious therapeutic challenges. In New York City, 22% of K. pneumoniae isolates are resistant to all commonly used antimicrobial agents,1 and carbapenem-resistant isolates of E. coli have been reported.3 Resistance to aminoglycosides is also common, particularly among K. pneumoniae, due to the acquisition of several aminoglycoside-modifying enzymes. Resistance to tigecycline and polymyxin B has also been noted in ~5% of isolates of K. pneumoniae.1 The development of new agents is sorely needed.

ACHN-490, a derivative of sisomicin, possesses activity against a broad range of Enterobacteriaceae, with MIC₉₀ values of ~1–2 mg/L.5–8 The activity of ACHN-490 is retained against most aminoglycoside-resistant isolates, including multidrug-resistant KPC-producing strains. ACHN-490 MICs do not seem to correlate with commonly found aminoglycoside-modifying enzymes. Although isolates in our study with increased ACHN-490 MICs were found to carry aac(6’)-Ib, this enzyme was also found in more susceptible strains. It is likely that the presence of a hydroxymethyl group at the 6’ position may stabilize this site against aac(6’)-Ib enzymes, and the occurrence of the enzyme in these isolates reflects its high prevalence in clinical isolates rather than any effect on ACHN-490 activity. Bactericidal activity is evident at concentrations 4× the MIC for most strains. However, regrowth has been found upon exposure to either ACHN-490 or traditional aminoglycosides in occasional isolates at this concentration.9 The mechanisms behind elevated MICs of ACHN-490 remain unclear. In Acinetobacter baumannii, increased expression of the efflux system AdeB may correlate with reduced susceptibility to ACHN-490. Although not encountered in this study, the presence of ribosomal methylases may affect susceptibility to this agent.8 Additional investigations involving Enterobacteriaceae, particularly studies examining the roles of efflux systems, ribosomal alterations and changes in outer membrane permeability, will be needed. Based on our in vitro results, ACHN-490 is a potentially useful therapeutic agent against multidrug-resistant E. coli and K. pneumoniae, including KPC-producing strains.

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Transparency declarations
None to declare.

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