ACHN-490, a Neoglycoside with Potent In Vitro Activity against Multidrug-Resistant *Klebsiella pneumoniae* Isolates

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The in vitro activity of ACHN-490, a novel aminoglycoside (“neoglycoside”), was evaluated against 102 multidrug-resistant (MDR) *Klebsiella pneumoniae* strains, including a subset of 25 strains producing the KPC carbapenemase. MIC₅₀ values for gentamicin, tobramycin, and amikacin were 8 μg/ml, 32 μg/ml, and 2 μg/ml, respectively; MIC₉₀ values for the same antimicrobials were ≥64 μg/ml, ≥64 μg/ml, and 32 μg/ml, respectively. ACHN-490 showed an MIC₅₀ of 0.5 μg/ml and an MIC₉₀ of 1 μg/ml, which are significantly lower than those of comparator aminoglycosides. ACHN-490 represents a promising aminoglycoside for the treatment of MDR *K. pneumoniae* isolates, including those producing KPC β-lactamase.

*Klebsiella pneumoniae* isolates producing extended-spectrum β-lactamases (ESBLs) represents a serious threat to our therapeutic armamentarium (21). These isolates are also frequently resistant to other classes of antibiotics, such as β-lactam/β-lactamase inhibitor combinations, quinolones, and aminoglycosides (8, 9), thereby limiting our choice to carbapenems for the treatment of serious infections (21).

Unfortunately, there is growing concern regarding the emergence of carbapenem-resistant *K. pneumoniae* isolates (20). In particular, *K. pneumoniae* isolates producing KPC carbapenemases (KPC-Kp) are spreading at an alarming rate in North and South America, the Caribbean, Europe, Israel, and Asia (6, 7, 15, 17, 18). Like ESBL producers, KPC-Kp are often resistant to quinolones and aminoglycosides (6). Therefore, our therapeutic options against KPC-Kp are limited to tigecycline and colistin. However, tigecycline may not reach desired serum levels to treat bloodstream infections (19), leaving colistin as the “last choice” against infections caused by KPC-Kp (13). Unfortunately, colistin-resistant KPC-Kp isolates are also reported in the United States (1, 12). As a result of this therapeutic dilemma, new antimicrobial agents with potent activity against multidrug-resistant (MDR) *K. pneumoniae* need to be developed.

Recently, there has been an increased interest in developing novel aminoglycosides. This new attention is due to (i) the potent bactericidal activity of aminoglycosides against a wide spectrum of aerobic gram-positive and gram-negative pathogens, (ii) the more gradual decline in susceptibility to aminoglycosides among gram-negative bacteria than that in susceptibility to other antimicrobials, and (iii) the ability of novel aminoglycosides to bypass common mechanisms of resistance that have gradually decreased the susceptibility to clinically used aminoglycosides (e.g., gentamicin, tobramycin, and amikacin) (11, 14, 16).

ACHN-490 (Achaogen, San Francisco, CA) is a “neoglycoside,” a next-generation aminoglycoside, currently in early clinical development (FDA, http://clinicaltrials.gov/), which has never been reported previously in the literature. The chemical structure of ACHN-490 is presented in Fig. 1.

In the present work, we analyzed the in vitro activity of ACHN-490 against a collection of 102 *K. pneumoniae* clinical isolates collected from January 2006 to October 2007 at the University of Pittsburgh Medical Center, and three Cleveland institutions, including University Hospitals Case Medical

![Chemical structure of ACHN-490](http://aac.asm.org/Downloaded-from-http://aac.asm.org)[/haba]-sisomicin].

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were interpreted according to the U.S. FDA criteria (i.e., guidelines recommended by CLSI (3)). Tigecycline MICs susceptibility results were interpreted according to the criteria, isolates were defined as ESBL producers when they showed a three twofold concentration decrease in MICs for ceftazidime or cefotaxime when tested alone (3).

MICs were determined by a microdilution method using cation-adjusted Mueller-Hinton broth, according to the Clinical and Laboratory Standards Institute (CLSI) criteria (2). Specific panels containing the following antibiotics were customized by Trek Diagnostics (Cleveland, OH): cefotaxime, cefotaxime-clavulanate (constant concentration of 4 mg/liter), ceftazidime, ceftazidime-clavulanate (constant concentration of 4 mg/liter), piperacillin-tazobactam, imipenem, ciprofloxacin, tigecycline, gentamicin, tobramycin, amikacin, arbekacin, neomycin, and ACHN-490. The following ATCC control strains were used: Escherichia coli 25922, Pseudomonas aeruginosa 27853, and K. pneumoniae 700603. Susceptibility results were interpreted according to the guidelines recommended by CLSI (3). Tigecycline MICs were interpreted according to the U.S. FDA criteria (i.e., susceptible at an MIC of ≤2 μg/ml). According to the CLSI criteria, isolates were defined as ESBL producers when they showed a three twofold concentration decrease in MICs for ceftazidime or cefotaxime when tested in combination with clavulanate versus their MICs when tested alone (3).

The 25 KPC-Kp isolates were analyzed by PCR for the presence of 16S rRNA methylase genes (i.e., *armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, and *nmpA*), using primers and conditions previously reported (4, 23). In addition, these strains were examined by PCR and sequencing for the presence of the most common aminoglycoside-modifying enzymes (AMEs) in gram-negative pathogens (22). In particular, the following genes were analyzed: *aac(6’)-Ib, aac(6’)-Ic, aac(6’)-Id, ant(3’)-Ia, ant(2’)-Ia, aac(3’)-Ia, aac(3’)-Ib, aac(3’)-Ic, aph(3’)-Vla, and aph(3’)-Vlb*, using primers previously reported (5, 10).

As shown in Table 1, MDR *K. pneumoniae* isolates were highly resistant to ceftazidime and piperacillin-tazobactam (each MIC\(\text{MIC}_{\text{MIC} \leq 2} \text{ μg/ml})

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>All MDR <em>K. pneumoniae</em> isolates (n = 102)</th>
<th>KPC-producing <em>K. pneumoniae</em> isolates (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC(\text{MIC}_{\text{MIC} \leq 2} \text{ μg/ml})</td>
<td>MIC(\text{MIC}_{\text{MIC} \leq 2} \text{ μg/ml})</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Neomycin</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ACHN-490</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

a: Susceptibility according to CLSI criteria (3); b: ceftazidime (MIC, ≤8 μg/ml); c: imipenem (MIC, ≤4 μg/ml); d: piperacillin-tazobactam (MIC, ≤16 μg/ml); e: cefotaxime-clavulanate (MIC, ≤1 μg/ml); amikacin (MIC, ≤16 μg/ml); gentamicin (MIC, ≤4 μg/ml); f: tobramycin (MIC, ≤4 μg/ml). g: CLSI criteria not available. h: E. coli ATCC 25922 (MICs, 0.5 to 1 μg/ml); P. aeruginosa ATCC 27853 (MIC, ≤4 μg/ml); K. pneumoniae ATCC 700603 (MICs, 0.25 to 0.5 μg/ml).

TABLE 1. Susceptibility results of MDR *K. pneumoniae* isolates, including those producing KPC enzymes.

For both MDR and KPC-Kp strains, ACHN-490 showed MIC\(\text{MIC}_{\text{MIC} \leq 2} \text{ μg/ml})

For both MDR and KPC-Kp strains, ACHN-490 showed MIC\(\text{MIC}_{\text{MIC} \leq 2} \text{ μg/ml})

To better understand the impact of these susceptibility data, we investigated the genetic background of KPC-Kp isolates in terms of their AMEs and methylases. All KPC-Kp strains were positive for *aac(6’)-Ib* and *ant(3’)-Ia* (alternative name of *aacA1*) AME genes. Since neither of these AMEs modifies gentamicin, this explains the lower level of gentamicin resistance observed in the KPC-Kp strains. In contrast, the AAC(3’)-II enzyme is common among *Enterobacteriaceae* and may be generating gentamicin resistance among the non-KPC-positive isolates (16). Two KPC-Kp strains (i.e., VA362 and VA373) were also positive for the *ant(2’)-Ia* gene. Consistent with our MIC results (i.e., all strains with arbekacin MICs of <32 μg/ml) and the low prevalence in the clinical population, we did not find any methylase genes. An *E. coli* control strain in which the *rmtA* methylase gene was cloned had an MIC of >8 μg/ml for ACHN-490.

In conclusion, ACHN-490 possesses potent in vitro activity against MDR *K. pneumoniae* isolates, including those producing KPC carbapenemase. ACHN-490 represents a promising alternative to tigecycline and colistin for the treatment of isolates resistant to quinolones, β-lactam/β-
FIG. 2. MIC distributions of amikacin, gentamicin, tobramycin, and ACHN-490 against the overall collection of MDR *K. pneumoniae* isolates (*n* = 102) and the subgroup of KPC-producing strains (*n* = 25). S, susceptible; I, intermediate; R, resistant. Results were interpreted according to CLSI criteria (3). Dashed vertical line, susceptibility cutoff; solid vertical line, resistance cutoff.
lactamase inhibitor combinations, carbapenems, and existing aminoglycosides.

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REFERENCES


