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

Andrea Endimiani,[1,2]* Kristine M. Hujer,[1,2] Andrea M. Hujer,[1] Eliana S. Armstrong,[3]
Yuvraj Choudhary,[1] James B. Aggen,[3] and Robert A. Bonomo[1,2,4,5]*

*Research Service, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, Ohio[1]; Department of
Medicine, Case Western Reserve University School of Medicine, Cleveland, Ohio[2]; Achaogen, Inc., South San Francisco,
California[3]; Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland, Ohio[4];
and Department of Molecular Biology and Microbiology, Case Western Reserve University School of
Medicine, Cleveland, Ohio[5]*

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. MIC_{50} values for gentamicin, tobramycin, and amikacin were 8 μg/ml, 32 μg/ml, and 2 μg/ml, respectively; MIC_{90} values for the same antimicrobials were ≥64 μg/ml, ≥64 μg/ml, and 32 μg/ml, respectively. ACHN-490 showed an MIC_{50} of 0.5 μg/ml and an MIC_{90} 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.

The spread of *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
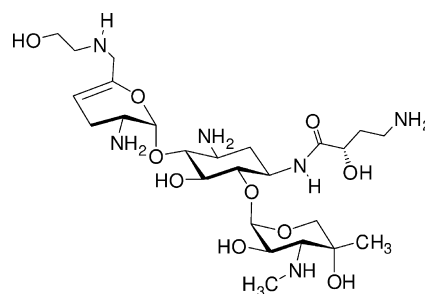
* Corresponding author. Mailing address: Infectious Diseases Section, Department of Veterans Affairs Medical Center, 10701 East Blvd., Cleveland, OH 44106 Phone: (216) 791-3800, ext. 4399. Fax: (216) 231-3482. E-mail for Robert A. Bonomo: robert.bonomo@va.gov. E-mail for Andrea Endimiani: aendimiani@gmail.com.

FIG. 1. Chemical structure of ACHN-490 [6′-(hydroxylethyl)-1-(haba)-sisomicin].

Center, the Cleveland Clinic, and the Louis Stokes Department of Veterans Affairs Medical Center.

The 102 *K. pneumoniae* isolates were selected based on an MDR phenotype (i.e., resistance to ≥3 antibiotic classes). Twenty-five isolates were KPC-Kp and were part of a previous study in which the β-lactamase background and clonality were characterized (6). The remaining 77 MDR *K. pneumoniae* isolates were ESBL producers, according to the phenotypic results (see below).

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 ≥3 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 *npmA*), 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)-IIc*, *aph(3')-VIa*, and *aph(3')-VIb*, 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$_{90}$, >32 μg/ml). Two-thirds of the isolates were resistant to ciprofloxacin, whereas approximately 75% and 90% of strains were still susceptible to imipenem and tigecycline, respectively. Almost all KPC-Kp isolates were resistant to β-lactams and quinolones, whereas tigecycline frequently remained active in vitro (Table 1). All of these 25 isolates were colistin susceptible, as previously reported (6).

Figure 2 shows our analysis of aminoglycoside susceptibility. MDR *K. pneumoniae* isolates were highly resistant to gentamicin and tobramycin (less than 26% of strains were susceptible). In contrast, amikacin still maintained in vitro activity (78% of isolates were susceptible) with only five isolates being fully resistant (i.e., MICs of 64 μg/ml). The subgroup of KPC-Kp showed lower susceptibility rates for amikacin and tobramycin (48% and 8%, respectively) than did the entire group of MDR strains (Fig. 2). Notably, gentamicin was more active in vitro against KPC-Kp (44%

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

Antibiotic	All MDR *K. pneumoniae* isolates (n = 102)			KPC-producing *K. pneumoniae* isolates (n = 25)		
	MIC$_{50}$ (μg/ml)	MIC$_{90}$ (μg/ml)	S (%)[a]	MIC$_{50}$ (μg/ml)	MIC$_{90}$ (μg/ml)	S (%)[a]
Ceftazidime	>32	>32	9.8	>32	>32	0.0
Imipenem	0.5	8	75.5	8	>16	12.0
Piperacillin-tazobactam	>64	>64	38.2	>64	>64	0.0
Ciprofloxacin	4	16	26.5	>8	>8	8.0
Tigecycline[b,c]	1	2	90.2	1	2	96.0
Amikacin	2	32	78.4	32	32	48.0
Gentamicin	8	≥64	25.5	8	16	44.0
Tobramycin	32	≥64	10.8	32	≥64	8.0
Arbekacin[c]	4	16		8	16	
Neomycin[c]	2	32		2	32	
ACHN-490[c,d]	0.5	1		0.5	1	

[a] S, susceptibility according to CLSI criteria (3): ceftazidime (MIC, ≤8 μg/ml); imipenem (MIC, ≤4 μg/ml); piperacillin-tazobactam (MIC, ≤16 μg/ml); ciprofloxacin (MIC, ≤1 μg/ml); amikacin (MIC, ≤16 μg/ml); gentamicin (MIC, ≤4 μg/ml); tobramycin (MIC, ≤4 μg/ml).
[b] Tigecycline was interpreted according to U.S. FDA criteria (susceptibility, MIC ≤ 2 μg/ml).
[c] CLSI criteria not available.
[d] *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).

of strains susceptible) than against the overall MDR isolate group.

For both MDR and KPC-Kp strains, ACHN-490 showed MIC$_{50}$ and MIC$_{90}$ values (i.e., 0.5 and 1 μg/ml, respectively) that were significantly lower than those for comparator aminoglycosides. The ACHN-490 MICs for all strains were ≤4 μg/ml. In particular, the MIC$_{90}$ of ACHN-490 was at least 5 twofold dilutions lower than that of amikacin, which is currently the aminoglycoside with the least resistance in our armamentarium (Fig. 2).

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 *aadA1*) 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-
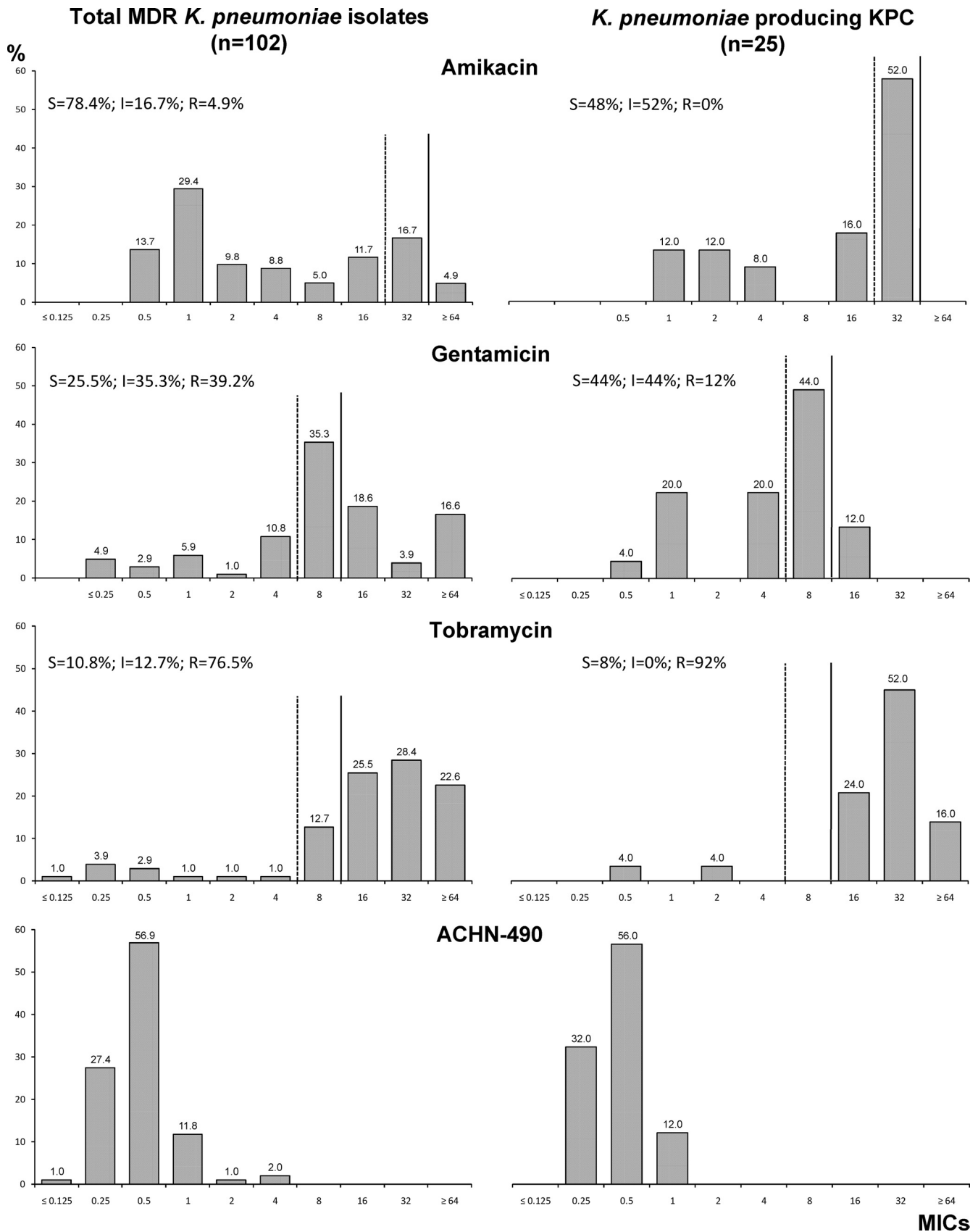
FIG. 2. MIC distributions of amikacin, gentamicin, tobramycin, and ACHN-490 against the overall collection of MDR *K. pneumoniae* isolates (*n* = 102) and the subgroup of KPC-producing strains (*n* = 25). S, susceptible; I, intermediate; R, resistant. Results were interpreted according to CLSI criteria (3). Dashed vertical line, susceptibility cutoff; solid vertical line, resistance cutoff.

lactamase inhibitor combinations, carbapenems, and existing aminoglycosides.

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