

Activity of ACHN-490, a Novel Neoglycoside Antibiotic, Against Contemporary Gram-Negative Clinical Isolates from Brooklyn, NY Hospitals.
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ABSTRACT

Background: MDR Gram-negative pathogens have become a widespread problem. We evaluated the activity of ACHN-490, a novel neoglycoside antibiotic, against a collection of contemporary Gram-negative clinical isolates.

Methods: Unique patient isolates of *P. aeruginosa* (PA), *A. baumannii* (AB), *K. pneumoniae* (KP), *E. coli* (EC), and *Enterobacter spp.* (EB) from prior surveillance studies at 16 Brooklyn, NY hospitals were selected based on resistance patterns. The majority of isolates were fingerprinted by automated ribotyping. MICs were performed by broth microdilution using CLSI standards. PCR was used to identify aminoglycoside-modifying enzymes (AMEs) in AB and KP and the KPC gene in Enterobacteriaceae. The expression of the efflux genes *mexA*, *mexC*, *mexE*, and *mexX* in PA, and *adeB* in AB was assessed by real-time RT-PCR.

Results: A total of 204 isolates were tested. Ribotyping revealed that 55% of isolates were unique strains. 45% of the PA and AB isolates were carbapenem-resistant, and 18% of the Enterobacteriaceae were KPC+. Susceptibility testing (Table) revealed that ACHN-490 had comparable activity to amikacin against PA and AB, and excellent activity against KP, EC, and EB, including amikacin-resistant and KPC+ strains. Among AB, isolates with the AME *aacA4* were more likely to have ACHN-490 MICs > 4 than those lacking *aacA4* (93% vs. 50%, P=0.01); however, some isolates lacking any AME achieved MICs > 16. Among AB, isolates with increased expression of *adeB* were more likely to have ACHN-490 MICs > 8 (69% vs. 13%, P<0.001). No relation was found between the presence of AMEs and ACHN-490 activity in KP isolates. Among PA, no relation was found between ACHN-490 activity and efflux gene expression.

		MIC ₅₀	MIC ₉₀	Susceptible
PA (n=33)	ACHN-490	8	16	
	Amikacin	4	16	94%
	Gentamicin	4	64	67%
	Imipenem	4	>8	55%
AB (n=38)	ACHN-490	8	>16	
	Amikacin	8	64	82%
	Gentamicin	16	>64	24%
	Imipenem	4	>8	55%
KP (n=71)	ACHN-490	0.5	1	
	Amikacin	16	64	58%
	Gentamicin	1	>64	59%
	Imipenem	0.25	>8	79%
EC (n=32)	ACHN-490	1	2	
	Amikacin	4	16	91%
	Gentamicin	1	64	72%
	Imipenem	0.125	4	91%
EB (n=30)	ACHN-490	1	4	
	Amikacin	4	16	93%
	Gentamicin	1	>64	70%
	Imipenem	0.5	2	93%
Ak-R-LF ^a (n=35)	ACHN-490	0.5	2	
KPC-LF ^b (n=24)	ACHN-490	0.5	4	

^a Amikacin-resistant lactose fermenters

^b KPC+-lactose fermenters

Conclusions: ACHN-490 shows promise as a novel agent against Enterobacteriaceae, including MDR-KPC+ strains.

INTRODUCTION

Multidrug-resistant (MDR) Gram-negative bacterial pathogens have become increasingly common in many medical centers around the world. While strains of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* have been problematic for many years, the carbapenem-hydrolyzing enzyme KPC has recently emerged among *Klebsiella pneumoniae* and other Enterobacteriaceae. The presence of the KPC gene on a plasmid-borne transposon has allowed for rapid spread of KPC to over 10 bacterial species on 4 continents. Strains possessing KPC, which affects all beta-lactam agents, typically harbor genes that confer resistance to fluoroquinolones, aminoglycosides (AGs), tetracyclines and trimethoprim-sulfamethoxazole. Many of these isolates are resistant to all currently available antibiotic agents. Clearly, there is an urgent need to develop antimicrobials with activity against MDR Gram-negative pathogens.

ACHN-490 is the first of a novel class of neoglycosides, or next-generation AGs, with enhanced activity against AG-resistant bacteria. We tested the in-vitro activity of ACHN-490 against a representative collection of clinical isolates of *P. aeruginosa*, *A. baumannii*, and Enterobacteriaceae from hospitals in Brooklyn, NY, an area endemic for MDR Gram-negative pathogens.

METHODS

Clinical isolates of *P. aeruginosa*, *A. baumannii*, *K. pneumoniae*, *E. coli*, and *Enterobacter spp.* were selected from surveillance studies conducted at 16 Brooklyn, NY hospitals between 1999 and 2006. Only one isolate per patient was included. Isolates were selected to approximate the AG susceptibility profile from the most recent surveillance study (J Antimicrob Chemother 2007;60:78) and to include representative samples of the major MDR clones in the region. Genetic fingerprinting of most isolates was performed by automated ribotyping (Qualicon, Wilmington, DE). MICs were done using the broth microdilution method according to CLSI standards. ACHN-490 was provided by Achaogen, Inc. (South San Francisco, CA).

PCR was used to screen all carbapenem-resistant isolates for *bla_{KPC}*, carbapenem-resistant *K. pneumoniae* and *A. baumannii* for the common AG-modifying enzymes (AMEs), and amikacin-resistant *A. baumannii* for the RNA methylases *armA*, *rmtA*, *rmtB*, *rmtC*, and *rmtD* as previously described (AAC 2008;52:2999). Real-time RT-PCR was performed as previously described to measure gene expression of the following proteins: *A. baumannii* - the OmpA-like porin and the efflux pumps AdeB and AbeM using ATCC19606 as control (AAC2008;52:2999); *P. aeruginosa* - the efflux pumps MexA, MexC, MexE and MexX using ATCC27853 as control (AAC2006; 50:1633, J Med Microbiol2007;56: 809); *K. pneumoniae* - the efflux pump AcrB using ATCC11296 as control (JAC2009;Epub). The relationship between gene expression and ACHN-490 MICs was tested by linear regression (Microsoft Excel), and between elevated ACHN-490 MICs and AG-modifying enzymes by Fisher's Exact test.

RESULTS

Ribotyping was performed on 84% of the 204 isolates, and revealed that 55% were unique strains. Results of MIC testing are shown in the Table.

Pseudomonas aeruginosa

Approximately 40% of isolates were resistant to ceftazidime and imipenem, and two thirds to ciprofloxacin. KPC was not found in any of the isolates. The activity of ACHN-490 was similar to amikacin, with 91% and 94% inhibited

	MIC ₅₀	MIC ₉₀	Range	%Susceptible
<i>P. aeruginosa</i> (n=33)				
ACHN-490	8	16	2->16	
Amikacin	4	16	1-64	94%
Gentamicin	4	64	<=0.5->64	67%
Imipenem	4	>8	1->8	55%
Ceftazidime	8	>16	1->16	61%
Piperacillin-tazobactam	4	>64	1->64	73%
Ciprofloxacin	8	>8	<=0.06->8	36%
Colistin	1	2	0.25-2	100%
<i>A. baumannii</i> (n=38)				
ACHN-490	8	>16	0.5->16	
Amikacin	8	64	<=0.5->64	82%
Gentamicin	16	>64	<=0.5->64	24%
Imipenem	4	>8	0.12->8	55%
Ceftazidime	>16	>16	4->16	21%
Piperacillin-tazobactam	>64	>64	1->64	18%
Ciprofloxacin	>8	>8	0.25->8	11%
Colistin	0.5	2	0.12-2	100%
Tigecycline	2	4	0.25->4	82%
<i>K. pneumoniae</i> (n=71)				
ACHN-490	0.5	1	<=0.12-2	
Amikacin	16	64	<=0.5->64	58%
Gentamicin	1	>64	<=0.5->64	59%
Imipenem	0.25	>8	<=0.06->8	79%
Ceftazidime	>16	>16	<=0.12->16	37%
Piperacillin-tazobactam	32	>64	<=0.5->64	49%
Ciprofloxacin	8	>8	<=0.06->8	47%
Colistin	0.25	1	0.06->4	97%
Tigecycline	0.25	2	0.06->4	90%
<i>E. coli</i> (n=32)				
ACHN-490	1	2	0.25-4	
Amikacin	4	16	<=0.5->64	91%
Gentamicin	1	64	<=0.5->64	72%
Imipenem	0.12	8	<=0.06->8	82%
Ceftazidime	1	4	<=0.12->16	69%
Piperacillin-tazobactam	2	>64	<=0.5->64	72%
Ciprofloxacin	>8	>8	<=0.06->8	31%
Colistin	0.12	2	0.06-2	100%
Tigecycline	0.12	4	0.06-4	97%
<i>Enterobacter sp</i> (n=30)				
ACHN-490	1	4	0.5-16	
Amikacin	4	16	1->64	94%
Gentamicin	1	4	<=0.5->64	70%
Imipenem	0.5	2	0.12->8	94%
Ceftazidime	>16	>16	<=0.12->16	27%
Piperacillin-tazobactam	64	>64	<=0.5->64	43%
Ciprofloxacin	0.12	1	<=0.06->8	74%
Colistin	0.25	1	0.12->4	97%
Tigecycline	0.5	4	0.25->4	87%

by ≤ 16 µg/ml, respectively. In a multivariate analysis, no relationship was found between the expression of the efflux genes *mexA*, *mexC*, *mexE* and *mexX* and ACHN-490 MICs.

Acinetobacter baumannii

Approximately half of isolates were resistant to carbapenems and over 80% to other beta-lactams and quinolones. The activity of ACHN-490 was similar to amikacin, with 89% and 82% inhibited by ≤ 16 µg/ml, respectively. Of note, 4 isolates with amikacin MICs of 32->64 µg/ml were inhibited by 8-16 µg/ml of ACHN-490. The genes for KPC and the RNA methylases were not found in any of the isolates. At least one AME was present in 2/3 of isolates. Isolates possessing the AME *aacA4* were more likely to have ACHN-490 MICs > 4 µg/ml than those lacking *aacA4* (93% vs. 50%, P=0.01). There was a direct correlation between expression of *adeB* and ACHN-490 MICs (P<0.001). However, some isolates with normal *adeB* expression and lacking any AME achieved MICs > 16, suggesting other resistance mechanisms. No relation was seen with *ompA* or *abeM* expression.

Enterobacteriaceae

21% of *K. pneumoniae*, 18% of *E. coli* and 6% of *Enterobacter spp.* were carbapenem-resistant. KPC was detected in 19% overall, including all carbapenem-resistant isolates. Approximately 2/3 were resistant to cephalosporins and half to quinolones. Only 19% of KPC+*K. pneumoniae* were susceptible to amikacin. Overall, 89% and 95% of Enterobacteriaceae were inhibited by 1 and 2 µg/ml of ACHN-490, respectively, regardless of amikacin susceptibility. Of 11 *K. pneumoniae* isolates that were resistant to all agents except colistin and/or tigecycline, all were inhibited by 0.25-1 µg/ml of ACHN-490. AMEs were detected in 28/35 *K. pneumoniae* isolates; the presence of these enzymes had no effect on ACHN-490 susceptibility. There was no relationship between expression of the efflux gene *acrB* and ACHN-490 susceptibility in *K. pneumoniae*.

CONCLUSIONS

1. ACHN-490, a novel neoglycoside antibiotic, shows excellent in-vitro activity against Enterobacteriaceae, including contemporary MDR-KPC-producing and AG-resistant strains.
2. The activity of ACHN-490 against *P. aeruginosa* and *A. baumannii* was similar to that of amikacin. Reduced susceptibility to ACHN-490 was associated with the gene for AG-modifying enzyme *aacA4* and expression of the efflux pump AdeB in some *A. baumannii* strains. Further study is needed to determine if there is a causal relationship between these factors and ACHN-490 susceptibility.
3. Additional studies will be needed to determine the clinical utility of ACHN-490 for Gram-negative infections, particularly due to MDR-strains.

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