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Antimicrobial Activity of ACHN-490, a Neoglycoside, Tested Against a Contemporary Collection of Clinical Isolates Including Problematic Antimicrobial-Resistant Phenotypes

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ABSTRACT

Background: ACHN-490 is the first neoglycoside, a next-generation aminoglycoside (AG) in clinical development. ACHN-490 activity was evaluated against Gram-negative (GN) isolates, *S. aureus* (SA) and Coagulase-negative staphylococci (CoNS) resistant (R) to current front-line antimicrobial agents.

Methods: 235 isolates were collected from medical centers worldwide and tested for susceptibility to ACHN-490 and comparator agents by CLSI broth microdilution methods. Enterobacteriaceae (ENT; n=125) included wildtype (WT) strains and those with ESBL, AmpC, KPC, NMC, SME and MBL enzymes. Non-ENT GN pathogens included WT *P. aeruginosa* (PSA) and *Acinetobacter* spp. (ACB) and carbapenem (CARB)-R strains, including those with MBL and OXA enzymes. SA and CoNS included oxacillin-susceptible (MSSA/MS-CoNS) and -R (MRSA/MR-CoNS) strains.

Results: The $MIC_{50/90}$ for ACHN-490 was ≤0.5/2, 8/32, 8/32, ≤0.5/1 and ≤0.5/≤0.5 µg/ml among ENT, PSA, ACB, SA and CoNS, respectively (Table). Although AG-R was not a selection criterion for this study, overall S to gentamicin and amikacin was only 66.0 and 77.0%, respectively. There were no differences in ACHN-490 potency against WT strains vs. isolates having R mechanisms with the exception of CARB-R PSA which were also less S to comparator AGs vs. WT strains. SA, including MRSA, and CoNS were readily inhibited by ACHN-490 (MICs, ≤2 µg/ml).

Organism/phenotype (no. Tested)	Cumulative % inhibited at ACHN-490 MIC								
	≤0.5	1	2	4	8	16	32	64	>64
Enterobacteriaceae (125)	56.0	80.0	92.0	98.4	98.4	98.4	98.4	98.4	100.0
WT (60)	41.7	70.0	88.3	100.0	-	-	-	-	-
ESBL (20)	65.0	90.0	95.0	95.0	95.0	95.0	95.0	95.0	100.0
KPC, NMC, SME (15)	60.0	100.0	-	-	-	-	-	-	-
AmpC (20)	75.0	85.0	95.0	95.0	95.0	95.0	95.0	95.0	100.0
MBL (10)	80.0	80.0	90.0	100.0	-	-	-	-	-
PSA (30)	0.0	3.3	3.3	30.0	50.0	76.7	96.7	96.7	100.0
WT (10)	0.0	10.0	10.0	70.0	100.0	-	-	-	-
CARB-R (20)	0.0	0.0	0.0	10.0	25.0	65.0	95.0	95.0	100.0
ACB (30)	3.3	10.0	23.3	26.7	66.7	70.0	90.0	100.0	-
WT (10)	0.0	0.0	10.0	20.0	60.0	60.0	80.0	100.0	-
CARB-R (20)	5.0	15.0	30.0	30.0	70.0	75.0	95.0	100.0	-
SA (30)	66.7	96.7	100.0	-	-	-	-	-	-
MSSA (10)	80.0	90.0	100.0	-	-	-	-	-	-
MRSA (20)	60.0	100.0	-	-	-	-	-	-	-
CoNS (20)	100.0	-	-	-	-	-	-	-	-

Conclusions: The remarkably consistent activity of ACHN-490 against leading GN pathogens, SA and CoNS, including those with increasingly prevalent R mechanisms is a promising feature of this novel agent.

INTRODUCTION

Aminoglycosides (AG) have a broad-spectrum of antimicrobial activity against aerobic Gram-positive and –negative bacterial pathogens. Gentamicin, tobramycin and amikacin are the most common AGs utilized for the treatment of bacterial pathogens in the United States (USA). Resistance mechanisms to these and other AGs include inactivation of the drugs by aminoglycoside modifying enzymes (AMEs), decreased permeability and/or upregulated efflux and less commonly, ribosomal alteration. The clinical importance of AMEs is significant as the encoding genes can be disseminated by plasmids or transposons. The AMEs include acetyltransferases, adenylyltransferases and phosphotransferases. ACHN-490 is a neoglycoside, or next-generation aminoglycoside, in clinical development that has been designed to overcome AG resistance mechanisms.

This study was conducted to determine the activity of ACHN-490 against common bacterial species that cause significant patient infections. Species included those with well known and established resistance such as methicillin-resistant *Staphylococcus aureus* (MRSA) as well as more recently recognized and/or evolving resistance mechanisms such as serine carbapenemases found in Gram-negative bacteria (e.g., KPC enzyme types). Enterobacteriaceae are common pathogens which have acquired numerous resistance mechanisms including Amp-C and extended spectrum β-lactamase (ESBL) enzyme production. Multi-drug resistance is common among staphylococci and non-fermentative Gram-negative bacilli such as *Pseudomonas aeruginosa* and *Acinetobacter* spp. Some endemic strains among each of these bacterial species have become so resistant that empiric therapy is problematic and antimicrobial therapy, even after the antibiogram is known, may be limited to only one class of compounds. Thus it is extremely important that the discovery of new antimicrobial classes or modifications to currently known agents be made to overcome evolving resistance problems.

MATERIALS AND METHODS

Isolates were tested by reference Clinical and Laboratory Standards Institute (CLSI) broth microdilution methods per M07-A8 [2009]. Antimicrobial susceptibility panels were manufactured by JMI Laboratories (North Liberty, Iowa, USA). Supplemental calcium (50 mg/L) was added to the broth media for testing daptomycin and freshly prepared broth media was utilized for testing tigecycline. Quality control (QC) strains included *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 29213. Interpretive criteria and QC ranges for comparator agents were as published in the CLSI M100-S19 [2009] document. Gram-negative isolates were tested against ACHN-490, amikacin, gentamicin, ceftazidime, piperacillin/tazobactam, imipenem, tigecycline, ciprofloxacin and polymyxin B. Staphylococci were tested against ACHN-490, amikacin, gentamicin, levofloxacin, linezolid, daptomycin, vancomycin and tigecycline.

Organisms (235 total) originated from patients in 88 medical centers participating in global surveillance programs in 2007 (28 countries). Isolates were identified to species level by two clinical microbiology laboratories including a reference laboratory (JMI Laboratories). Seventy-four isolates of species with unusual resistance phenotypes were isolated prior to 2007. Organisms demonstrated various antibiogram profiles, including some strains with resistance to at least one or more marketed aminoglycoside class agents. The Gram-negative organism collection with phenotype/genotype characterizations is listed in Table 1. Staphylococci included oxacillin-susceptible and -resistant isolates as well as community-acquired strains.

RESULTS

- No significant differences in ACHN-490 potency were noted between wild-type enteric isolates and those that produced various β-lactamase/AmpC/carbapenemase enzymes (Table 2).
- Species of Proteae had slightly higher MIC values than other Enterobacteriaceae and one isolate each of *Enterobacter* spp. and *Klebsiella* spp. had a MIC value of >64 µg/ml (also resistant to gentamicin and amikacin). These two isolates were later demonstrated to possess ribosomal methyltransferases (armA and rmtB).
- Among all Enterobacteriaceae isolates, $MIC_{50/90}$ results for ACHN-490 were ≤0.5 and 2 µg/ml, respectively. With the exception of two isolates, all organisms were inhibited by ≤4 µg/ml (Tables 2 and 3). ACHN-490 was the most potent agent tested in this study and was eight-fold and ≥32-fold more active than amikacin and gentamicin, respectively. Resistance to other antimicrobial classes ranged from 9.6 - 41.6%.
- Regardless of enzyme production or resistance mechanism, all but one isolate of *P. aeruginosa* had ACHN-490 MIC values between 1 and 32 µg/ml (Table 2). MIC_{50} and MIC_{90} values of 8 and 32 µg/ml were observed for all isolates combined (Table 4). One isolate (MβL-producer) had a MIC value of >64 µg/ml.
- Against *Acinetobacter* spp., no differences in ACHN-490 activity were observed between carbapenem-susceptible and -resistant isolates (Table 2) with MIC_{50} and MIC_{90} values of 8 and 32 µg/ml (Table 4).
- Significant resistance was noted for other antimicrobial classes against the non-enteric Gram-negative pathogens, including aminoglycosides at 36.7 – 70.0% (Table 4).
- Regardless of oxacillin susceptibility pattern, *S. aureus* was readily inhibited by ACHN-490 (MIC_{90} values, ≤1 µg/ml) with all isolates inhibited by ≤2 µg/ml (Table 5). This investigational agent readily inhibited coagulase-negative staphylococci (CoNS); all isolates were inhibited by ≤0.5 µg/ml (Table 5).

Table 1. Characterization of the Gram-negative bacterial pathogens tested in this study.

Organism	Category (no.)	Enzyme(s)
E. coli	Wild-Type (10)	
	Plasmidic AmpC (6)	CMY-2, FOX-5
	ESBL (10)[a]	CTX-M, OXA, TEM
	Carbapenemase (1)	KPC-3
Klebsiella spp.	Wild-Type (10)	
	ESBL (10)[a]	CTX-M, OXA, SHV, TEM
	Carbapenemase (12)	IMP, VIM, KPC-2, KPC-3
Enterobacter spp.	Wild-Type (5)	
	Derepressed AmpC (5)	
	Carbapenemase (6)	IMP, VIM, KPC-2, KPC-3, NMC-A
Citrobacter spp.	Wild-Type (5)	
	Derepressed AmpC (5)	
	Carbapenemase (2)	VIM, KPC-3
Serratia spp.	Wild-Type (10)	
	Carbapenemase (3)	KPC-3, SME-2
Proteae	Wild-Type (20)	
	Plasmidic AmpC (1)	DHA-1
	Carbapenemase (1)	VIM
Salmonella spp.	Plasmidic AmpC (3)	CMY-2
P. aeruginosa	Wild-Type (10)	
	Carbapenem-resistant (10)	
	Carbapenemase (10)	GIM, IMP, SPM, VIM
Acinetobacter spp.	Wild-Type (10)	
	Oxacillinase (10)[a]	OXA-23, -24, -51, -58
	Carbapenemase (10)	IMP

a. Multiple enzymes were often present.

Table 2. MIC distributions of ACHN-490 when tested against common bacterial pathogens, including resistant subsets.

Organism group (no. tested)[a]	Number inhibited at each MIC (µg/ml)								
	≤0.5	1	2	4	8	16	32	64	>64
E. coli (20)	9	8	3	-	-	-	-	-	-
Wild-Type (10)	5	3	2	-	-	-	-	-	-
ESBL-producers (10)	4	5	1	-	-	-	-	-	-
Klebsiella spp. (20)	17	1	1	-	-	-	-	-	1
Wild-type (10)	8	1	1	-	-	-	-	-	-
ESBL-producers (10)	9	-	-	-	-	-	-	-	1
Enterobacter spp. (10)	8	1	-	-	-	-	-	-	1
Wild-type (5)	5	-	-	-	-	-	-	-	-
Ceftazidime-resistant (5)	3	1	-	-	-	-	-	-	1
Citrobacter spp. (10)	10	-	-	-	-	-	-	-	-
Wild-type (5)	5	-	-	-	-	-	-	-	-
Ceftazidime-resistant (5)	5	-	-	-	-	-	-	-	-
Serratia spp. (10)	2	8	-	-	-	-	-	-	-
P. mirabilis (10)	-	1	3	6	-	-	-	-	-
Indole-positive Proteae (10)	-	4	5	1	-	-	-	-	-
Serine-carbapenemase-positive enterics (15)	9	6	-	-	-	-	-	-	-
Plasmidic AmpC-positive enterics (10)	7	1	2	-	-	-	-	-	-
Metallo-β-lactamase (MβL)-positive enterics (10)	8	-	1	1	-	-	-	-	-
P. aeruginosa (30)	-	1	-	8	6	8	6	-	1
Wild-type (10)	-	1	-	6	3	-	-	-	-
Carbapenem-resistant, MβL-negative (10)	-	-	-	2	1	4	3	-	-
Carbapenem-resistant, MβL-positive (10)	-	-	-	-	2	4	3	-	1
Acinetobacter spp. (30)	1	2	4	1	12	1	6	3	-
Wild-Type (10)	-	-	1	1	4	-	2	2	-
Carbapenem-resistant, MβL-positive (10)	-	1	2	-	3	1	3	-	-
Oxacillinase-producers (10)	1	1	1	-	5	-	1	1	-
S. aureus (30)	20	9	1	-	-	-	-	-	-
Oxacillin-susceptible (10)	8	1	1	-	-	-	-	-	-
Oxacillin-resistant (20)	12	8	-	-	-	-	-	-	-
Coagulase-negative staphylococci (20)	20	-	-	-	-	-	-	-	-
Oxacillin-susceptible (10)	10	-	-	-	-	-	-	-	-
Oxacillin-resistant (10)	10	-	-	-	-	-	-	-	-

a. For species composition of these groups see Table 1.

Table 3. Comparison of in vitro activity of ACHN-490 and selected antimicrobial agents tested against Enterobacteriaceae (125 strains).

Antimicrobial agent	MIC_{50}	MIC_{90}	MIC Range	% susceptible/resistant[a]
ACHN-490	≤0.5	2	≤0.5 – >64	- / -
Gentamicin	0.5	>32	≤0.25 – >32	76.0 / 18.4
Amikacin	2	16	≤0.5 – >64	92.0 / 4.0
Ceftazidime	4	>32	≤0.25 – >32	55.2 / 41.6
Imipenem	0.5	8	≤0.12 - >16	87.2 / 9.6
Piperacillin/tazobactam	2	>64	≤0.5 – >64	72.0 / 18.4
Ciprofloxacin	≤0.06	>8	≤0.06 – >8	69.6 / 28.8
Tigecycline[b]	0.12	0.12	0.06 – 4	97.6 / -
Polymyxin B	1	>8	0.5 – >8	- / -

a. Criteria as published by the CLSI [2009].
b. USA-FDA breakpoints were applied [Tygacil Product Insert, 2005].

Table 4. Comparison of in vitro activity of ACHN-490 and selected antimicrobial agents tested against non-enteric Gram-negative pathogens (60 strains).

Organism/antimicrobial agent	MIC_{50}	MIC_{90}	MIC Range	% susceptible/resistant[a]
P. aeruginosa (30)				
ACHN-490	8	32	1 - >64	- / -[b]
Gentamicin	8	>32	1 - >32	46.7 / 40.0
Amikacin	16	>64	1 - >64	60.0 / 36.7
Ceftazidime	>32	>32	2 - >32	10.0 / 83.3
Imipenem	16	>16	0.5 - >16	36.7 / 56.7
Piperacillin/tazobactam	64	>64	2 - >64	53.3 / 46.7
Ciprofloxacin	2	>8	0.12 - >8	40.0 / 46.7
Tigecycline[b]	>4	>4	1 - >4	- / -
Polymyxin B	2	2	0.5 - 8	93.3 / 3.3
Acinetobacter spp. (30)				
ACHN-490	8	32	≤0.5 - 64	- / -
Gentamicin	32	>32	0.5 - >32	16.7 / 66.7
Amikacin	64	>64	2 - >64	23.3 / 70.0
Ceftazidime	>32	>32	4 - >32	3.3 / 93.3
Imipenem	>16	>16	≤0.12 - >16	33.3 / 66.7
Piperacillin/tazobactam	>64	>64	≤0.5 - >64	13.3 / 66.7
Ciprofloxacin	>8	>8	0.12 - >8	23.3 / 73.3
Tigecycline[b]	1	2	0.06 -4	- / -
Polymyxin B	1	2	0.5 - >8	90.0 / 10.0

a. Criteria as published by the CLSI [2009].
b. No susceptibility breakpoint criteria have been recommended.

Table 5. Comparison of in vitro activity of ACHN-490 and selected antimicrobial agents tested against staphylococci (50 strains).

Organism/antimicrobial agent	MIC_{50}	MIC_{90}	MIC Range	% susceptible/resistant[a]
S. aureus (30)[b]				
ACHN-490	0.5	1	≤0.5 - 2	- / -
Gentamicin	≤0.25	>32	≤0.25 - >32	86.7 / 13.3
Amikacin	8	32	2 - >64	73.3 / 6.7
Levofloxacin	0.25	>4	0.06 - >4	73.3 / 26.7
Vancomycin	1	1	0.5 – 1	100.0 / 0.0
Daptomycin	0.25	0.5	0.25 – 1	100.0 / -
Linezolid	2	2	2	100.0 / -
Tigecycline[c]	0.06	0.12	0.06 – 0.25	100.0 / -
CoNS (20)[d]				
ACHN-490	≤0.5	≤0.5	≤0.5	- / -
Gentamicin	≤0.25	>32	≤0.25 - >32	75.0 / 20.0
Amikacin	1	4	≤0.5 - >64	95.0 / 5.0
Levofloxacin	0.12	>4	0.06 - >4	55.0 / 45.0
Vancomycin	1	2	0.5 – 2	100.0 / 0.0
Daptomycin	0.25	0.5	0.12 – 0.5	100.0 / -
Linezolid	0.5	1	0.25 – 1	100.0 / -
Tigecycline[c]	0.06	0.25	≤0.03 – 0.25	- / -

a. Criteria as published by the CLSI [2009].
b. 66.7% of isolates were resistant to oxacillin.
c. USA-FDA breakpoints were applied [Tygacil Product Insert, 2005].
d. 50.0% of isolates were resistant to oxacillin.

CONCLUSIONS

- Overall, ACHN-490 displayed activity against all tested pathogens including Enterobacteriaceae (MIC_{90}, 2 µg/ml), *P. aeruginosa* (32 µg/ml), *Acinetobacter* spp. (32 µg/ml), *S. aureus* (1 µg/ml) and CoNS (≤0.5 µg/ml).
- The presence of extended spectrum β-lactamases, chromosomal or plasmidic AmpC cephalosporinases, serine carbapenemases and metallo-β-lactamases had no effect on MIC values of ACHN-490.
- The remarkably consistent activity against leading Gram-negative and -positive pathogens, including those with increasingly prevalent resistant mechanisms, is a promising feature of ACHN-490. MIC_{50} and MIC_{90} potencies suggest that the majority of isolates may be inhibited by achievable concentrations of this novel agent pending appropriate pharmacokinetic/ pharmacodynamic and target attainment studies.

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