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MECHANISMS OF RESISTANCE

ARGONAUT II Study of the *In Vitro* Activity of Plazomicin against Carbapenemase-Producing *Klebsiella pneumoniae*

Michael R. Jacobs,[a,b] Caryn E. Good,[a,b] Andrea M. Hujer,[c,d] Ayman M. Abdelhamed,[a,b] Daniel D. Rhoads,[a,b] Kristine M. Hujer,[c,d] Susan D. Rudin,[c,d] T. Nicholas Domitrovic,[c,d] Lynn E. Connolly,[e] Kevin M. Krause,[e] Robin Patel,[f] Cesar A. Arias,[g,h,i,j] Barry N. Kreiswirth,[k] Laura J. Rojas,[c,d] Roshan D'Souza,[l] Richard C. White,[l] Lauren M. Brinkac,[l] Kevin Nguyen,[l] Indresh Singh,[l] Derrick E. Fouts,[l] David van Duin,[m] Robert A. Bonomo,[c,d,n,o,p,q,r] for the Antibacterial Resistance Leadership Group

[a]Department of Pathology, Case Western Reserve University, Cleveland, Ohio, USA
[b]Department of Pathology, University Hospitals Cleveland Medical Center, Cleveland, Ohio, USA
[c]Department of Medicine, Case Western Reserve University, Cleveland, Ohio, USA
[d]Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, Ohio, USA
[e]Achaogen, Inc., South San Francisco, California, USA
[f]Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA
[g]Division of Infectious Diseases, McGovern School of Medicine at Houston, Houston, Texas, USA
[h]Center for Antimicrobial Resistance and Microbial Genomics, McGovern School of Medicine at Houston, Houston, Texas, USA
[i]Center for Infectious Diseases, UTHealth School of Public Health, Houston, Texas, USA
[j]Molecular Genetics and Antimicrobial Resistance Unit, International Center for Microbial Genomics, Universidad El Bosque, Bogota, Colombia
[k]Hackensack Meridian Health Center for Discovery and Innovation, Nutley, New Jersey, USA
[l]J. Craig Venter Institute, Rockville, Maryland, USA
[m]Division of Infectious Diseases, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA
[n]Department of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, Ohio, USA
[o]Department of Pharmacology, Case Western Reserve University, Cleveland, Ohio, USA
[p]Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio, USA
[q]Center for Proteomics and Bioinformatics, Case Western Reserve University, Cleveland, Ohio, USA
[r]CWRU-Cleveland VAMC Center for Antimicrobial Resistance and Epidemiology, Cleveland, Ohio, USA

ABSTRACT Plazomicin was tested against 697 recently acquired carbapenem-resistant *Klebsiella pneumoniae* isolates from the Great Lakes region of the United States. Plazomicin MIC_{50} and MIC_{90} values were 0.25 and 1 mg/liter, respectively; 680 isolates (97.6%) were susceptible (MICs of ≤2 mg/liter), 9 (1.3%) intermediate (MICs of 4 mg/liter), and 8 (1.1%) resistant (MICs of >32 mg/liter). Resistance was associated with *rmtF*-, *rmtB*-, or *armA*-encoded 16S rRNA methyltransferases in all except 1 isolate.

KEYWORDS *Klebsiella*, antibiotic resistance, carbapenemase, plazomicin

Antimicrobial resistance is recognized as a major challenge by the World Health Organization and the U.S. Centers for Disease Control and Prevention, with carbapenem resistance in multidrug-resistant Gram-negative pathogens being of particular concern (1, 2). The most recent U.S. report notes that the burden of resistance is greater than initially estimated, documenting that there are now more than 2.8 million antibiotic-resistant infections in the United States each year, with over 35,000 fatalities (3).

A new aminoglycoside, plazomicin, was recently approved by the Food and Drug Administration (FDA) for the management of complicated urinary tract infections and pyelonephritis (4). Plazomicin is active against most aminoglycoside-resistant *Enterobacteriaceae* strains but is not uniformly active against *Pseudomonas aeruginosa* or

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Address correspondence to Michael R. Jacobs, mrj6@case.edu, or Robert A. Bonomo, robert.bonomo@va.gov.

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TABLE 1 MIC ranges, MIC_{50} values, and MIC_{90} values, with percentages of isolates susceptible based on CLSI/FDA breakpoints

Agent	MIC range (mg/liter)	MIC_{50} (mg/liter)	MIC_{90} (mg/liter)	% susceptible
Plazomicin	≤0.12 to >32	0.25	1	97.6
Gentamicin	≤0.5 to >32	4	>32	53.3
Tobramycin	≤0.25 to >32	32	>32	14.3
Amikacin	≤1 to >64	16	32	64.1
Ceftriaxone	≤1 to >8	>8	>8	0.6
Ceftazidime	≤0.5 to >8	>8	>8	0.9
Aztreonam	≤1 to >16	>16	>16	0.9
Imipenem	≤0.25 to >8	>8	>8	3.3
Meropenem	≤0.12 to >8	>8	>8	4.6
Doripenem	≤0.12 to >8	8	>8	5.4
Piperacillin-tazobactam	4/4 to >64/4	>64/4	>64/4	0.4
Levofloxacin	≤0.25 to >4	>4	>4	4.1
Trimethoprim-sulfamethoxazole	≤1/19 to >4/76	>4/76	>4/76	20.6
Colistin	0.5 to >8	0.5	>8	85.3[a]
Tigecycline	0.5 to >4	1	4	87.6

[a]Percentage based on EUCAST breakpoint.

Acinetobacter baumannii (5). The Consortium on Resistance Against Carbapenems in *Klebsiella* and Other *Enterobacteriaceae* (CRACKLE), part of the Antibacterial Resistance Leadership Group (ARLG), is a federally funded, prospective multicenter consortium that tracks carbapenem-resistant *Enterobacteriaceae* (CRE) strains. This analysis is the second in a series of ARLG Reference Group for the Testing of Novel Therapeutics (ARGONAUT) studies, in which the *in vitro* activities of plazomicin and comparators were evaluated against a CRACKLE collection of carbapenemase-producing *Klebsiella pneumoniae* clinical isolates collected from the Great Lakes region between 2012 and 2016 (6).

Plazomicin MICs against a collection of 697 carbapenem-resistant *K. pneumoniae* isolates with defined carbapenem resistance mechanisms (6) were determined by broth microdilution assays, according to current Clinical and Laboratory Standards Institute (CLSI) guidelines (7). Testing was performed using custom frozen panels (Thermo Fisher) containing plazomicin and comparator agents. MICs were interpreted according to FDA and CLSI breakpoints, with plazomicin MICs being interpreted as follows: susceptible, ≤2 mg/liter; intermediate, 4 mg/liter; resistant, ≥8 mg/liter. Resistance mechanisms of plazomicin-intermediate and resistant isolates were characterized by whole-genome sequencing (WGS; BioProject accession numbers PRJNA433394 and PRJNA339843) (6). Briefly, WGS was performed using paired-end Nextera XT libraries on an Illumina NextSeq (2 × 150 bp) to 100-fold coverage. Reads were assembled using SPAdes, annotated with the NCBI Prokaryotic Genome Annotation Pipeline, and used to determine resistome, plasmid types, and multilocus sequence type through ResFinder 3.2, PlasmidFinder 2.1, and MLST 2.0, respectively, from the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/index.html).

Carbapenemase genes present in the 697 study isolates included bla_{KPC-2} ($n = 323$), bla_{KPC-3} ($n = 364$), bla_{KPC-4} or $bla_{KPC-4-like}$ ($n = 2$), $bla_{OXA-48-like}$ ($n = 7$), and bla_{NDM-5} and $bla_{OXA-48-like}$ ($n = 1$). Antimicrobial susceptibility findings are shown in Table 1. Fewer than 1% of isolates were susceptible to ceftriaxone, ceftazidime, aztreonam, or piperacillin-tazobactam, while ≤5.4% were susceptible to imipenem, meropenem, or doripenem. Most isolates (95.9%) were also resistant to levofloxacin. Susceptibility to other agents was more varied, with 20.6% being susceptible to trimethoprim-sulfamethoxazole, 87.6% to tigecycline, 14.3% to tobramycin, 53.3% to gentamicin, and 64.1% to amikacin. Plazomicin MICs ranged from ≤0.12 to >32 mg/liter, with MIC_{50} and MIC_{90} values of 0.25 and 1 mg/liter, respectively. Overall, 680 isolates (97.6%) were susceptible (MICs of ≤2 mg/liter), while 9 (1.3%) were intermediate and 8 (1.1%) were resistant (MICs of >32 mg/liter) to plazomicin.

The 8 plazomicin-resistant isolates were also resistant to gentamicin, tobramycin, and amikacin; they were collected from hospitals in Ohio between 2014 and 2016. They were obtained from abscess ($n = 3$), urinary ($n = 2$), respiratory ($n = 2$), and wound

TABLE 2 Genes coding for resistance to antimicrobial agents, as detected by WGS of plazomicin-resistant isolates

ST, plasmids, and resistance genes detected	ARLG-2713	ARLG-2756[a]	ARLG-2757[a]	ARLG-2881[b]	ARLG-2882[b]	ARLG-3126	ARLG-3135	ARLG-3143
ST	ST16	ST147	ST147	ST395	ST395	ST231	ST231	ST231
Plasmids	IncFIB(pQil), IncFII(K), IncFIB(K)	IncFII(Pkpx1), IncFII, ColpVC	IncFII(Pkpx1), IncFII, ColpVC	IncL/M(pOXA-48), IncFIB(Mar), IncR, IncHI1B	IncL/M(pOXA-48), IncFIB(Mar), IncR	IncFIB(pQil), IncFIA, Col4401, ColKP3, IncFII(K), IncFII(pAMA1167-NDM-5), Col(BS512)	IncFIB(pQil), IncFIA, Col4401, ColKP3, IncFII(K), IncFII(pAMA1167-NDM-5)	IncFIB(pQil), IncFIA, Col4401, ColKP3, IncFII(K), IncFII(pAMA1167-NDM-5), Col(BS512)
16S rRNA methyltransferase genes	*rmtF*	*rmtB*, *rmtF*	*rmtF*	*armA*	None	*rmtF*	*rmtF*	*rmtF*
β-Lactamase genes	bla_{OXA-1}, bla_{SHV-1}, $bla_{CTX-M-15}$[c]	$bla_{OXA-181}$, bla_{SHV-11}, bla_{TEM-1}, $bla_{CTX-M-15}$, bla_{NDM-5}	$bla_{OXA-181}$, bla_{SHV-11}, $bla_{CTX-M-15}$	bla_{OXA-48}, bla_{SHV-11}, bla_{TEM-1}	bla_{OXA-48}, bla_{SHV-11}, bla_{TEM-1}	$bla_{OXA-232}$, bla_{SHV-1}, bla_{TEM-1}, $bla_{CTX-M-15}$[c]	$bla_{OXA-232}$, bla_{SHV-1}, bla_{TEM-1}, $bla_{CTX-M-15}$	$bla_{OXA-232}$, bla_{SHV-1}, bla_{TEM-1}, $bla_{CTX-M-15}$[c]
Genes for resistance to								
Aminoglycosides	*aadA2*	*aadA2*, *aac(6′)-Ib*	*aac(6′)-Ib*	None	None	*aac(6′)-Ib*	*aadA2*, *aac(6′)-Ib*	*aadA2*, *aac(6′)-Ib*
Fluoroquinolones	*gyrA* D87N, *parC* E84K, *parE* S458T	*parC* S80I, *qnrB1*[c]	*parC* S80I, *qnrB1*[c]	*parC* S80I, *qnrS1*	*parC* S80I, *qnrS1*	*parC* S80I, *qnrS1*	*parC* S80I, *qnrS1*	*parC* S80I, *qnrS1*
Macrolides, lincosamides, streptogramin B	*mph*(A), *mrx*	*mph*(A), *mrx*, *erm*(B)	None	*msr*(E), *mph*(E)	None	None	*mph*(A), *erm*(B)	*mph*(A), *mrx*, *erm*(B)
Chloramphenicol	*catB4*[c]	None	None	*catA1*	*catA1*	*catA1*	*catA1*	*catA1*
Rifampin	*arr-2*	*arr-2*	*arr-2*	None	None	*arr-2*	*arr-2*	*arr-2*
Sulfonamides	*sul1*	*sul1*	None	*sul1*	None	None	*sul1*	*sul1*
Tetracyclines	*tet*(A)	None	None	*tet*(A)	None	None	None	None
Trimethoprim	*dfrA12*	*dfrA12*, *dfrA14*	*dfrA14*	*dfrA1*	None	None	*dfrA12*	*dfrA12*

[a]ARLG-2756 and ARLG-2757 are isolates from the same patient (11).
[b]ARLG-2881 and ARLG-2882 are isolates from the same patient.
[c]Incomplete gene present.

($n = 1$) cultures from 6 unique patients. These patients (4 male patients and 2 female patients) had a median age of 55 years (range, 25 to 69 years). Four patients were admitted from long-term-care facilities. One patient was admitted from home, and 1 was transferred from a hospital abroad. Five patients were admitted to the intensive care unit (ICU), with a median ICU stay of 6 days (range, 2 to 22 days). Five patients required mechanical ventilation and 3 patients underwent tracheostomy prior to the first positive CRE culture. Patients were treated with various antibiotics, sometimes in combination; 4 patients received tigecycline, 3 meropenem, 2 inhaled colistin, and 1 each ceftazidime-avibactam, gentamicin, amikacin, and trimethoprim-sulfamethoxazole. All patients survived their hospitalizations, with 4 being discharged to long-term-care facilities and 2 discharged home.

WGS of the 8 plazomicin-resistant isolates showed that the strains each carried several plasmids, had multiple antibiotic resistance determinants, and belonged to four multilocus sequence types (STs) (Table 2). The STs included ST16 ($n = 1$, associated with $bla_{\text{OXA-1}}$), ST147 ($n = 2$, associated with $bla_{\text{OXA-181}}$), ST395 ($n = 2$, associated with $bla_{\text{OXA-48}}$), and ST231 ($n = 3$, associated with $bla_{\text{OXA-232}}$). The isolates belonging to ST147, ST395, and ST231 harbored the same or very similar plasmids and resistance mechanisms associated with each ST (Table 2). Plazomicin resistance was associated with 16S rRNA methyltransferases (a known mechanism of resistance) in 7 of the 8 isolates (8). The 16S rRNA methyltransferase genes included *rmtF* in the ST16, ST147, and ST231 isolates (1 ST147 isolate also carried *rmtB*) and *armA* in 1 of the ST395 isolates. Known plazomicin resistance mechanisms could not be identified in the other ST395 isolate (ARLG-2882). Other studies have reported ST147 and ST231 as *K. pneumoniae* STs that carry *rmt* genes (9–12). Interestingly, 16S rRNA methyltransferase genes were not found by WGS in 8 of the 9 plazomicin-intermediate isolates (1 isolate was not sequenced).

Plazomicin resistance is frequently associated with strains harboring New Delhi metallo-β-lactamases (NDMs) in other countries (12–15). However, NDM is not commonly encountered in the United States (13), and only 1 of the 8 plazomicin-resistant strains in our study possessed NDM-mediated carbapenem resistance (Table 2).

Plazomicin is a recently approved next-generation aminoglycoside agent with activity against carbapenemase-producing *Enterobacteriaceae* strains, with potency comparable to that of ceftazidime-avibactam, meropenem-vaborbactam, and imipenem-relebactam (16). Our results compare favorably with a recently published study in which 94.9% of 117 bla_{KPC}-positive isolates were susceptible to plazomicin, compared to 43.6%, 56.4%, and 5.1% susceptible to amikacin, gentamicin, and tobramycin, respectively (17). Although plazomicin resistance is infrequent in the United States, of concern is the occurrence of *armA* and *rmtF* 16S rRNA methyltransferase genes in 7 of our plazomicin-resistant isolates, a finding noted in other recent studies (8, 13–15, 18). Our study documents the *in vitro* activity of plazomicin against a large U.S. collection of carbapenemase-producing *K. pneumoniae* isolates, with 97.6% of isolates being susceptible to this agent.

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