ASM Microbe 2017
Sunday - 9

Aminoglycoside-Resistance Genes Among 2014–2015 US Carbapenem-Resistant *Enterobacteriaceae* Isolates and Activity of Plazomicin against Characterized Isolates

M CASTANHEIRA[1], LN WOOSLEY[1], TB DOYLE[1], AW SERIO[2], KM KRAUSE[2], RK FLAMM[1]

[1]JMI Laboratories, North Liberty, Iowa, USA; [2]Achaogen, South San Francisco, California, USA

Contact Information:
Mariana Castanheira, Ph.D.
JMI Laboratories
345 Beaver Kreek Centre, Suite A
North Liberty, IA 52317
Phone: (319) 665-3370
Fax: (319) 665-3371
Email: mariana-castanheira@jmilabs.com

Abstract

Background: Plazomicin (PLZ) is a next generation aminoglycoside (AMG) developed to overcome common aminoglycoside-resistance mechanisms and has completed Phase 3 studies in complicated urinary tract infections and serious infections due to carbapenem-resistant *Enterobacteriaceae* (CRE). We evaluated the activity of PLZ and clinically available aminoglycosides against CRE isolates collected in US hospitals.

Methods: Among 4,362 *Enterobacteriaceae* isolates collected in US hospitals during 2014–2015 and susceptibility (S) tested by CLSI reference broth microdilution methods, 97 were CRE (CLSI criteria). CRE isolates were screened for carbapenemase-encoding genes by PCR/sequencing. Isolates displaying non-S MICs (CLSI criteria) for gentamicin (GEN), amikacin (AMK), and/or tobramycin (TOB) were screened for aminoglycoside-modifying enzymes (*aac(6')-Ib*, *aac(3)-IIa*, *ant(2'')-Ia*, *aph(3')-VIa*, *aac(3)-I*-like, and *aac(3)-IVa*). Isolates displaying PLZ MIC results at ≥128 µg/mL were tested for 16S rRNA methylases (RNAmet).

Results: Among 97 CRE, 31 KPC-2, 57 KPC-3, and 1 KPC-17 were detected and 8 isolates were negative for carbapenemase genes tested. Non-S MIC values for at least 1 of the clinically available AMG were noted for 81 CRE isolates. AME genes alone or in combination were detected among 77 isolates. The most common genes were *acc(6')-Ib*, *aac(3)-IVa*, and *aac(3)-IIa* (72, 16 and 10 isolates, respectively). Combinations of these genes included *aac(6')-Ib* with *aac(3)-IVa* (13 isolates), *aac(3)-IIa* (8), or *ant(2'')-Ia* (2). PLZ inhibited 99.0% of CRE isolates at ≤2 or ≤4 µg/mL. AMK, GEN, and TOB were active against 64.9%, 56.7%, and 13.4% (CLSI criteria) of the CRE isolates, respectively. The highest PLZ MIC value for isolates producing AMEs was 2 µg/mL. AMK, GEN, and TOB were active against 59.7%, 49.4%, and 0.0% of AME-producing isolates. One isolate displayed PLZ MIC >2 µg/mL (MIC, >128 µg/mL); this isolate carried the RNAmet *rmtF* and was resistant to all clinically available AMGs.

Conclusion: PLZ was active against CRE isolates from US hospitals, including 77 isolates carrying AMEs that were resistant to clinically available AMGs. PLZ is a potential treatment for serious infections caused by CRE where treatment options are limited.

Organism/group (no. tested)	Cumulative % at PLZ MIC (µg/mL)			
	0.5	1	2	4
CRE (97)	82.5	96.9	99.0	99.0
AME producers (77)	81.8	97.4	100.0	
aac(6')-Ib (72)	81.9	97.2	100.0	
aac(3)-IVa (16)	68.8	87.5	100.0	
aac(3)-IIa (10)	100.0			

Introduction

- Carbapenem-resistant *Enterobacteriaceae* (CRE) isolates have been detected worldwide, and their increasing prevalence is mainly due to the dissemination of isolates producing carbapenemases
- KPC-, VIM-, and NDM-variants have spread globally amongst various *Enterobacteriaceae* species
- Carbapenemase-producing *Enterobacteriaceae* (CPE) isolates are resistant to all or nearly all β-lactam agents and are often resistant to other antimicrobial classes, limiting therapeutic options to treat these infections
- The multidrug-resistant nature of CPE often limits available treatment options for infections caused by these isolates to tigecycline and/or colistin; however, their usage has limitations and resistance to these agents is increasingly reported worldwide
- Plazomicin is a semi-synthetic aminoglycoside that retains activity against CPE including most aminoglycoside resistant isolates
- We evaluated the activity of plazomicin and clinically available aminoglycosides against CRE isolates collected in US hospitals during 2014 and 2015 and genetically characterized the aminoglycoside resistance mechanisms among these isolates

Materials and Methods

- A total of 4,362 unique *Enterobacteriaceae* clinical isolates were included in the study
- Isolates collected during 2014–2015 from 30 US hospitals were susceptibility tested using the reference broth microdilution method described by the Clinical and Laboratory Standards Institute (CLSI)
- Categorical interpretations for all comparator agents were those found in CLSI criteria in M100-S27 (2017), EUCAST breakpoint tables (version 7.0, January 2017), and/or United States Food and Drug Administration (US FDA) package inserts
- Quality control (QC) was performed according to CLSI guidelines (M7-A9), and all QC MIC results were within acceptable ranges as published in CLSI documents
- CRE was defined as any isolate exhibiting doripenem, imipenem, and/or meropenem MIC values at ≥2 µg/mL
  - *Proteus mirabilis* and indole-positive Proteeae were categorized as CRE if doripenem and/or meropenem MIC values were at ≥2 µg/mL due to intrinsically elevated imipenem MIC values
- CRE isolates were screened for acquired carbapenemase encoding genes by PCR using custom primers for bla_{KPC}, bla_{SME}, bla_{GES}, bla_{NMC-A}, bla_{IMI}, bla_{IMP}, bla_{VIM}, bla_{SPM-1}, bla_{GIM-1}, bla_{SIM-1}, bla_{AIM-1}, bla_{KHM-1}, bla_{NDM}, bla_{DIM-1}, bla_{BIC-1}, bla_{BCK-1} and bla_{FRI-1}
- *Enterobacteriaceae* isolates displaying plazomicin MIC results at ≥128 µg/mL were screened for the presence of 16S rRNA methylases encoding genes *rmtA-H, armA*, and *npmA*
- Amplicons for carbapenemases and 16S rRNA methylase encoding genes were sequenced on both strands, and nucleotide sequences obtained were analyzed using the Lasergene® software package (DNAStar; Madison, Wisconsin, USA) and compared to available sequences via NCBI BLAST search (http://www.ncbi.nlm.nih.gov/blast/)
- *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., and *Enterobacter* spp. isolates displaying nonsusceptible MIC values for gentamicin, amikacin, and/or tobramycin (CLSI criteria) and with plazomicin MIC values ≤64 µg/mL were screened for the presence of the following AME genes: *aac(6')-Ib*, *aac(3)-IIa*, *ant(2'')-Ia*, *aph(3')-VIa* and *aac(3)-Ia, -Ib, -Ic, -Id, -Ie*, and *aac(3)-IVa*
  - Isolates that had any AME gene amplified in the multiplex reaction were retested by PCR using the appropriate individual primer pairs for confirmation

Results

- Among 4,362 *Enterobacteriaceae* isolates collected from US hospitals during 2014 and 2015, 97 (2.2%) were CRE and 89 carried genes encoding KPC (31 KPC-2, 57 KPC-3, and 1 KPC-17)
  - The other 8 CRE isolates were negative for all carbapenemases tested
- AME genes were detected in 77 of 81 CRE that were non-susceptible to one or more of the clinically available aminoglycosides
  - AME genes detected were *acc(6')-Ib* (72 isolates), *aac(3)-IVa* (16), *aac(3)-IIa* (10), and *ant(2'')-Ia* (2; Table 1)
  - Combinations of these genes included *aac(6')-Ib* plus *aac(3)-IVa* (13 isolates), *aac(6')-Ib* plus *aac(3)-IIa* (8), and *aac(6')-Ib* plus *ant(2'')-Ia* (2)
- Only 1 CRE isolate displayed plazomicin MIC results at ≥128 µg/mL and was additionally tested for 16S RNA methylase encoding genes. This isolate was a KPC-3-producing *K. pneumoniae*-carrying *rmtF*
- Plazomicin ($MIC_{50/90}$, 0.5/1 µg/mL) was very active against CRE isolates, including the 77 isolates carrying AME genes, and inhibited 99.0% of these isolates at ≤2 or ≤4 µg/mL (Table 2 and Figure 1)
  - Amikacin ($MIC_{50/90}$, 16/32 µg/mL), gentamicin ($MIC_{50/90}$, 8/>8 µg/mL), and tobramycin ($MIC_{50/90}$, >8/>8 µg/mL) displayed reduced activity against isolates carrying AME genes, inhibiting 59.7%, 49.4% and 0.0%, respectively, of the isolates at current CLSI breakpoints (Table 2)
- The activity of plazomicin ($MIC_{50/90}$ ranges, 0.12 to 0.5/0.5 to 1 µg/mL) was not affected by the type of AME gene detected among CRE isolates or the presence of multiple AME genes (Table 2)
- Plazomicin activity was stable regardless of the bla_{KPC} variant carried ($MIC_{50/90}$ ranges, 0.25 to 0.5/1 µg/mL) or absence of carbapenemase (carbapenemase-negative isolates; MIC_{50}, 0.25 µg/mL; Table 2)
- The isolate carrying *rmtF* was the only isolate displaying a plazomicin MIC value >2 µg/mL (MIC, >128 µg/mL) and was highly resistant to all aminoglycosides tested

Table 1 AME genes detected among CRE isolates collected in US hospitals from 2014 to 2015

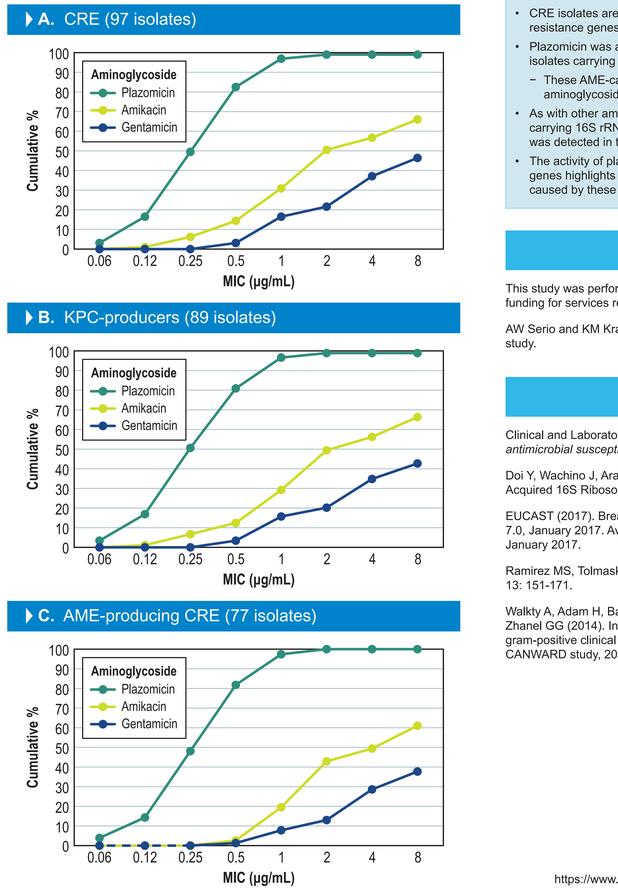
Organism/ organism groups	No. tested	No of positive results:					No of isolates positive for:						No. tested negative
		aac(3)-IIa	*aac(3)-IVa*	*aac(6')-Ib*	*ant(2'')-Ia*		*aac(3)-IIa* only	*aac(3)-IVa* only	*aac(6')-Ib* only	*aac(3)-IVa, aac(6')-Ib*	*aac3-IIa, aac(6')-Ib*	*ant(2'')-Ia, aac(6')-Ib*	
All CRE	**97**	**10**	**16**	**72**	**2**		**2**	**3**	**49**	**13**	**8**	**2**	**19**
C. freundii	1												1
E. aerogenes	1												1
E. cloacae	2			2	1				1			1	
K. oxytoca	4			3	1				2			1	1
K. pneumoniae	87	10	16	67			2	3	46	13	8		14
S. marcescens	2												2

Table 2 Activity of plazomicin and comparator aminoglycoside agents tested against CRE isolates carrying AME genes

Carbapenemase groups antimicrobial agent	(Number of isolates positive) MIC_{50}/MIC_{90} (µg/mL)							
		Gene*				Combinations		
	Any AME	*aac(6')-Ib*	*aac(3)-IVa*	*aac(3)-IIa*	*ant(2'')-Ia*	*aac(3)-IVa, aac(6')-Ib*	*aac(3)-IIa, aac(6')-Ib*	*aac(6')-Ib, ant(2'')-Ia*
All CRE (97)	**(77)**	**(72)**	**(16)**	**(10)**	**(2)**	**(13)**	**(8)**	**(2)**
Plazomicin	0.5 / 1	0.5 / 1	0.5 / 2	0.25 / 0.5	0.12 / -	0.5 / 2	0.25 / -	0.12 / -
Amikacin	16 / 32	16 / 32	16 / 32	8 / 32	4 / -	16 / 32	32 / -	4 / -
Gentamicin	8 / >8	4 / >8	>8 / >8	>8 / >8	>8 / -	>8 / >8	>8 / -	>8 / -
Tobramycin	>8 / >8	>8 / >8	>8 / >8	>8 / >8	>8 / -	>8 / >8	>8 / -	>8 / -
All KPC (89)	**(72)**	**(68)**	**(15)**	**(7)**	**(2)**	**(12)**	**(6)**	**(2)**
Plazomicin	0.5 / 1	0.5 / 1	0.5 / 2	0.25 / -	0.12 / -	0.5 / 2	0.25 / -	0.12 / -
Amikacin	16 / 32	16 / 32	16 / 32	32 / -	4 / -	16 / 32	32 / -	4 / -
Gentamicin	4 / >8	4 / >8	>8 / >8	>8 / -	>8 / -	>8 / >8	2 / -	>8 / -
Tobramycin	>8 / >8	>8 / >8	>8 / >8	>8 / -	>8 / -	>8 / >8	>8 / -	>8 / -
KPC-2 (31)	**(25)**	**(23)**	**(7)**	**(3)**	**(2)**	**(6)**	**(2)**	**(2)**
Plazomicin	0.25 / 1	0.25 / 1	0.5 / -	0.25 / -	0.12 / -	0.25 / -	0.12 / -	0.12 / -
Amikacin	16 / 32	16 / 32	16 / -	2 / -	4 / -	16 / -	2 / -	4 / -
Gentamicin	>8 / >8	>8 / >8	>8 / -	>32 / -	>8 / -	>8 / -	2 / -	>8 / -
Tobramycin	>8 / >8	>8 / >8	>8 / -	>8 / -	>8 / -	>8 / -	>8 / -	>8 / -
KPC-3 (57)	**(47)**	**(45)**	**(8)**	**(4)**	**(0)**	**(6)**	**(4)**	**(0)**
Plazomicin	0.5 / 1	0.5 / 1	0.5 / -	0.25 / -		0.5 / -	0.25 / -	
Amikacin	16 / >32	16 / >32	16 / -	32 / -		16 / -	32 / -	
Gentamicin	4 / >8	4 / >8	>8 / -	2 / -		>8 / -	2 / -	
Tobramycin	>8 / >8	>8 / >8	>8 / -	>8 / -		>8 / -	>8 / -	
Carbapenemase negative (8)	**(5)**	**(4)**	**(1)**	**(3)**	**(0)**	**(1)**	**(2)**	**(0)**
Plazomicin	0.25 / -	0.25 / -	- / -	0.25 / -		- / -	0.25 / -	
Amikacin	8 / -	8 / -	- / -	4 / -		- / -	4 / -	
Gentamicin	>32 / -	2 / -	- / -	>32 / -		- / -	>32 / -	
Tobramycin	>8 / -	>8 / -	- / -	>8 / -		- / -	>8 / -	

*Includes all isolates carrying the gene, including those that might have multiple AME genes

Figure 1 Frequency distributions of plazomicin and comparator aminoglycoside agents tested against CRE isolates



Conclusions

- CRE isolates are usually resistant to aminoglycosides due to the presence of resistance genes in the same genetic elements
- Plazomicin was active against CRE isolates from US hospitals, including 77 (79.4%) isolates carrying 1 or more AME genes
  - These AME-carrying CRE isolates displayed elevated MIC values for other aminoglycosides
- As with other aminoglycosides, plazomicin displayed no activity against isolates carrying 16S rRNA methylase genes; however, only 1 isolate carrying these genes was detected in this 2-year CRE collection from the US
- The activity of plazomicin against CRE isolates that usually carry 1 or more AME genes highlights the importance of further developing this agent to treat infections caused by these isolates

Acknowledgments

This study was performed by JMI Laboratories and supported by Achaogen, which included funding for services related to preparing this poster.

AW Serio and KM Krause are employees of Achaogen and contributed to the design of the study.

References

Clinical and Laboratory Standards Institute (2017). *M100-S27. Performance standards for antimicrobial susceptibility testing: 27th informational supplement.* Wayne, PA: CLSI.

Doi Y, Wachino J, Arakawa Y (2016). Aminoglycoside Resistance: The Emergence of Acquired 16S Ribosomal RNA Methyltransferases. *Infect Dis Clin North Am* 30: 523-537.

EUCAST (2017). Breakpoint tables for interpretation of MICs and zone diameters. Version 7.0, January 2017. Available at: http://www.eucast.org/clinical_breakpoints/. Accessed January 2017.

Ramirez MS, Tolmasky ME (2010). Aminoglycoside modifying enzymes. *Drug Resist Updat* 13: 151-171.

Walkty A, Adam H, Baxter M, Denisuik A, Lagace-Wiens P, Karlowsky JA, Hoban DJ, Zhanel GG (2014). In vitro activity of plazomicin against 5,015 gram-negative and gram-positive clinical isolates obtained from patients in Canadian hospitals as part of the CANWARD study, 2011-2012. *Antimicrob Agents Chemother* 58: 2554-2563.

https://www.jmilabs.com/data/posters/ASMMicrobe17-plazo-CRE-US.pdf