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Activity of Plazomicin against *Enterobacteriaceae* Isolates Collected in the United States Including Isolates Carrying Aminoglycoside-Modifying Enzymes Detected by Whole Genome Sequencing

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Abstract

Background: Plazomicin (PLZ) is a next-generation aminoglycoside (AMG) stable against aminoglycoside-modifying enzymes (AME) that completed Phase 3 studies for complicated urinary tract infections and serious infections due to carbapenem-resistant *Enterobacteriaceae* (ENT). We evaluated the activity of PLZ and AMGs against ENT collected in US hospitals during 2016.

Methods: A total of 2,097 ENT were susceptibility (S) tested by CLSI reference broth microdilution methods. *E. coli*, *Klebsiella* spp., *Enterobacter* spp., and *P. mirabilis* isolates displaying non-S MICs (CLSI criteria) for gentamicin (GEN), amikacin (AMK), and/or tobramycin (TOB) were submitted to WGS, *de novo* assembly and screening for AME genes.

Results: Against ENT, PLZ was more active than all 3 clinically available AMGs. PLZ and AMK activities were stable regardless of the infection type; however, differences were observed for GEN and TOB. Bloodstream isolates displayed higher GEN MICs when compared to the other infection sites. TOB activity varied 4-fold, being higher for bloodstream and pneumonia infections and lower for skin and skin structure tissue and other/unknown specimens. Against 198 isolates carrying 1 or more AME-encoding genes detected among 208 AMG-non-S isolates, PLZ activity was 8- to 16-fold greater when compared to the activity of AMK and at least 16-fold higher than the activity of GEN or TOB.

Conclusions: PLZ was active against ENT isolates from US hospitals regardless of infection type. PLZ displayed activity against isolates carrying AME genes that represent 12.0% of selected species. AME-carrying isolates were considerably more resistant to AMK, GEN, and TOB, highlighting the potential value of PLZ to treat infections caused by these organisms.

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Introduction

- Plazomicin is a semi-synthetic aminoglycoside derived from sisomicin and contains structural modifications that allow it to retain activity in the presence of the most prevalent aminoglycoside-modifying enzymes (AMEs)
- Aminoglycoside modification and inactivation mediated by AMEs are the most common resistance mechanisms to aminoglycoside agents in gram-positive and -negative bacteria
- AMEs are grouped according to the type of modification that they catalyze and are aminoglycoside acetyltransferases (AACs), nucleotidyltransferases, or adenyltransferases (ANTs) and phosphotransferases (APHs)
- We evaluated the activity of plazomicin and clinically available aminoglycosides against 2,097 *Enterobacteriaceae* isolates collected in United States (US) hospitals during 2016 and characterized the aminoglycoside resistance mechanisms among these isolates using whole genome sequencing analysis

Materials and Methods

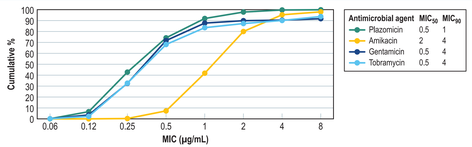
- A total of 2,097 *Enterobacteriaceae* isolates deemed as cause of infection and limited to 1 per patient episode were included in the study
- Isolates collected during 2016 from 30 US hospitals were susceptibility tested using the reference broth microdilution method described by the Clinical and Laboratory 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 (M07-A9), and all QC MIC results were within acceptable ranges as published in CLSI documents
- A total of 208 *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., and *Enterobacter* spp. isolates displaying nonsusceptible MIC values for gentamicin, amikacin, and/or tobramycin according to CLSI criteria were screened for the presence of AME and 16S rRNA methylase encoding genes
  - Selected isolates were submitted to whole genome sequencing on a MiSeq (Illumina, San Diego, California, US) instrument targeting a 30X coverage
  - Sequences were *de novo* assembled and searched for the presence of AME and 16S rRNA methylase-encoding genes using a curated library and applying criteria of >94% sequencing identity and 40% minimum length coverage

Results

- Plazomicin ($MIC_{50/90}$, 0.5/1 µg/mL) inhibited 97.8% and 99.7% of the 2,097 *Enterobacteriaceae* isolates from US hospitals collected during 2016 at ≤2 µg/mL and ≤4 µg/mL, respectively (Figure 1)
  - Only 7 isolates displayed plazomicin MIC values >4 µg/mL (8–32 µg/mL): 3 *Morganella morganii*, 3 *Providencia stuartii,* and 1 *Providencia rettgeri*
- Applying CLSI/EUCAST breakpoints, gentamicin ($MIC_{50/90}$, 0.5/4 µg/mL) inhibited 90.4/89.9%, amikacin ($MIC_{50/90}$, 2/4 µg/mL) inhibited 99.3/98.0%, and tobramycin ($MIC_{50/90}$, 0.5/4 µg/mL) inhibited 90.1/87.3% of these isolates, respectively (data not shown)
- The activity of plazomicin (MIC_{50} ranges 0.25 to 0.5 µg/mL; MIC_{90} ranges 1 to 2 µg/mL) and amikacin were not affected by infection site; however, differences were observed for gentamicin and tobramycin (Figure 1)
  - Bloodstream isolates displayed a higher gentamicin MIC_{90} value (>8 µg/mL) when compared to the other infection sites (1–4 µg/mL)
  - Bloodstream and pneumonia isolates displayed higher tobramycin MIC_{90} values (8 µg/mL) when compared to skin and skin structure tissue and other/unknown specimens (MIC_{90}: 2 µg/mL)

Figure 1 Plazomicin and comparator aminoglycoside agents tested against *Enterobacteriaceae* isolates collected in US hospitals during 2016

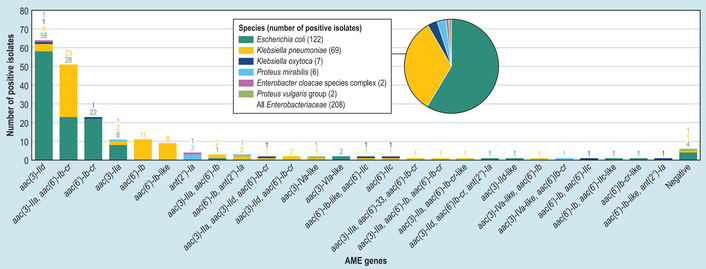
A. All infection sites (2,097 tested)



B. Frequency distributions of plazomicin and comparator agents against *Enterobacteriaceae* isolates

	MIC50/90 (µg/mL)			
Organism group/infection type (no. tested)	**Plazomicin**	**Amikacin**	**Gentamicin**	**Tobramycin**
Enterobacteriaceae (2,097)	0.5/1	2/4	0.5/4	0.5/4
Urinary tract infection (587)	0.5/1	2/4	0.5/2	0.5/4
Bloodstream infection (572)	0.5/1	2/4	0.5/>8	0.5/8
Pneumonia in hospitalized patients (451)	0.25/1	2/4	0.5/4	0.5/8
Skin and skin structure infection (298)	0.5/2	2/4	0.5/1	0.5/2
Intra-abdominal infection (152)	0.5/1	2/4	0.5/4	0.5/4
Other sites (37)	0.25/1	1/2	0.5/1	0.5/2
Isolates carrying AME genes (198)	0.5/1	4/16	>8/>8	>8/>8

Figure 2 AME genes that modify broadly used aminoglycosides detected among *Enterobacteriaceae* isolates collected in US hospitals during 2016



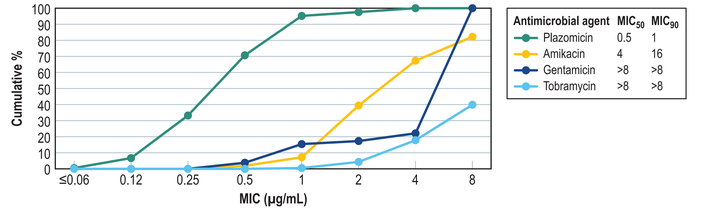
- AME genes detected were among all 208 (12.0% of the species tested) isolates tested, and 202 isolates carried genes that are known to encode resistance to 1 or more broadly used aminoglycosides (amikacin, gentamicin, and tobramycin; Figure 2)
  - The most common genes detected were *aac(6')-Ib-cr* (82 isolates), *aac(3)-IIa* (70), *aac(3)-IId* (69), and *aac(6')-Ib* (21)
  - The combination *aac(3)-IIa* plus *aac(6')-Ib-cr* was observed among 51 isolates, and 14 other combinations of 2 or 3 of these genes were also observed (Figure 2)
- Plazomicin was very active against isolates carrying AME genes, and the highest plazomicin MIC against isolates carrying AME genes was 4 µg/mL (Figure 3)
  - Overall, plazomicin ($MIC_{50/90}$, 0.5/1 µg/mL) had 8- to 16-fold more potent activity when compared to amikacin ($MIC_{50/90}$, 4/16 µg/mL) and at least 16-fold more potent activity than gentamicin and tobramycin ($MIC_{50/90}$, >8/>8 µg/mL for both)
- The activity of amikacin, gentamicin, and tobramycin varied against isolates carrying different AME genes alone or in combination (Figures 3B to 3D)
- None of the isolates tested carried genes encoding 16S rRNA methylases

Conclusions

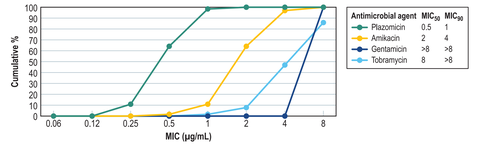
- Plazomicin was active against *Enterobacteriaceae* isolates from US hospitals regardless of infection type
- Plazomicin displayed activity against isolates carrying AME genes, including the genes that encode resistance to amikacin, gentamicin, and tobramycin
- Isolates carrying 16S rRNA methylases were not observed in this collection and remain rare in US hospitals
- AMEs were detected in approximately 10.0% of the overall isolates and 12.0% of the species tested
  - These isolates are common and usually resistant to the currently available aminoglycosides
  - Plazomicin displayed good activity against these isolates regardless of the gene or genes detected

Figure 3 Frequency distributions of plazomicin and comparator aminoglycoside agents tested against AME-carrying isolates

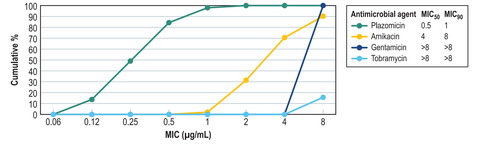
A. All AME-carrying isolates (208 detected)



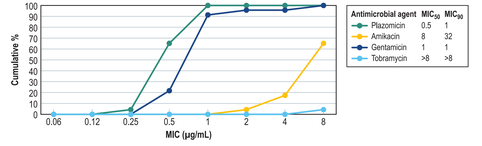
B. *aac(3)-IId* (64 detected)



C. *aac(3)-IIa, aac(6')-Ib-cr* (51 detected)



D. *aac(6')Ib-cr* (23 detected)



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