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Plazomicin Activity against *Enterobacteriaceae* Collected from Europe, Latin America, and Asia-Pacific during 2016, Including Those with Aminoglycoside and Beta-Lactam Resistance Mechanisms

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Amended Abstract

Background: Plazomicin, a next-generation aminoglycoside, was developed to overcome common aminoglycoside-resistance mechanisms. We evaluated plazomicin activity against *Enterobacteriaceae* clinical isolates collected in Europe (n=2,045), Latin America (n=511), and Asia-Pacific (n=682) during 2016 and evaluated aminoglycoside and beta-lactam resistance mechanisms among these isolates.

Materials/methods: A total of 3,238 *Enterobacteriaceae* were susceptibility tested using the reference broth microdilution method. ESBL-phenotype, carbapenem-resistant *Enterobacteriaceae* (CRE), and isolates resistant to ≥1 aminoglycoside were screened for resistance genes using whole genome sequencing analysis.

Results: Plazomicin ($MIC_{50/90}$, 0.5/1 mg/L) inhibited 96.3% and 98.2% of the *Enterobacteriaceae* at ≤2 mg/L and ≤4 mg/L, respectively. Amikacin, gentamicin, and tobramycin inhibited 94.3%, 82.1%, and 75.9% of these isolates, respectively (EUCAST breakpoints). Plazomicin displayed activity against *E. coli* (n=1,182; $MIC_{50/90}$, 0.5/1 mg/L), *K. pneumoniae* (n=1,115; $MIC_{50/90}$, 0.25/0.5 mg/L), and *E. cloacae* species complex (n=120; $MIC_{50/90}$, 0.25/0.5 mg/L). Plazomicin ($MIC_{50/90}$, 0.25/1 mg/L) inhibited 94.7% and 94.8% of the 688 isolates carrying ESBL genes at ≤2 mg/L and ≤4 mg/L, respectively. The most common ESBL genes were $bla_{CTX-M-15}$ (n=486) and $bla_{CTX-M-14}$ (n=51). Plazomicin inhibited 78.8% of the 170 CRE at ≤2 mg/L or ≤4 mg/L. Other aminoglycosides inhibited 14.1% to 48.8% of these isolates (EUCAST breakpoints). Carbapenemase genes were found in 138 CRE isolates and included 74 bla_{KPC}, 39 bla_{OXA-48}-like, and 26 bla_{NDM-1}. Aminoglycoside-modifying enzymes (AME) were observed among 630/644 isolates tested and the most common genes were *aac(6')-Ib-cr* (n=353) and *aac(3)-IIa* (n=312). Plazomicin ($MIC_{50/90}$, 0.5/2 mg/L) inhibited 92.7% and 93.5% of the AME-carrying isolates at ≤2 mg/L and ≤4 mg/L, respectively. Amikacin, gentamicin, and tobramycin inhibited 75.1%, 17.5%, and 1.7%, respectively, of these isolates using the EUCAST breakpoints. 16S rRNA methylases were detected in 49 isolates that were resistant to all AMGs and had plazomicin MIC values >128 mg/L. One *Providencia stuartii* isolate carried *aac(2')-Ia* and displayed plazomicin MIC values of ≥128 mg/L.

Conclusions: Plazomicin was active against the *Enterobacteriaceae* tested, including ESBL- and AME-carrying isolates and approximately 80% of the CRE. Our results support the development plan for plazomicin to treat serious infections caused by resistant *Enterobacteriaceae* when treatment options are limited.

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Introduction

- Plazomicin is a semi-synthetic aminoglycoside developed from sisomicin that demonstrates activity against *Enterobacteriaceae*, including multidrug-resistant isolates, *Staphylococcus* spp., and some *Pseudomonas aeruginosa*
- Plazomicin contains structural modifications that allow it to retain activity in the presence of aminoglycoside-modifying enzymes (AMEs)
  - AMEs are the most common resistance mechanism to aminoglycoside agents in gram-positive and -negative bacteria and confer resistance by aminoglycoside modification and subsequent inactivation
- Plazomicin has been evaluated in 2 phase 3 clinical trials, including a study to evaluate the efficacy of this aminoglycoside against carbapenem-resistant *Enterobacteriaceae* (CRE)
- We evaluated the activity of plazomicin and comparator antimicrobial agents against 3,238 *Enterobacteriaceae* isolates collected in hospitals located in Europe, Asia-Pacific, and Latin America as part of the Antimicrobial Longitudinal Evaluation of Resistance Trends (ALERT) program
  - Isolates displaying resistance to 1 or more aminoglycosides or resistance to broad-spectrum β-lactams were evaluated for resistance mechanisms against these antimicrobial classes using whole genome sequencing analysis

Materials and Methods

- A total of 3,238 *Enterobacteriaceae* isolates collected during 2016 from 62 hospitals and identified as causative of infection were included in the study; isolates were limited to 1 per patient episode and were included in the ALERT Program
- Isolates 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 in the EUCAST breakpoint tables (version 7.0, January 2017)
  - Quality control (QC) was performed according to CLSI guidelines (M07), and all QC MIC results were within acceptable ranges as published in CLSI documents
- Extended-spectrum beta-lactamase (ESBL)-phenotype was defined as an MIC at ≥2 mg/L for ceftriaxone, ceftazidime, or aztreonam (CLSI, 2018) for *Escherichia coli*, *K. pneumoniae* and *K. oxytoca*, and *Proteus mirabilis*
- CRE was defined as any isolate exhibiting doripenem, imipenem, and/or meropenem MIC values at ≥2 mg/L
  - *Proteus mirabilis* and indole-positive Proteeae were categorized as CRE if doripenem and/or meropenem MIC values were at ≥2 mg/L due to intrinsically elevated imipenem MIC values
- Whole genome sequencing on a MiSeq (Illumina, San Diego, California, US) instrument targeting a 30X coverage was performed on selected isolates
  - *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 AMEs and any *Enterobacteriaceae* isolate with plazomicin MIC values of ≥128 mg/L was screened for AMEs and 16S rRNA methyltransferase encoding genes
  - CRE and *Enterobacteriaceae* isolates displaying an ESBL-phenotype were screened for the presence of β-lactamases
- Sequences were *de novo* assembled and genes encoding resistance were searched using a curated library and applying criteria of >94% sequencing identity and 40% minimum length coverage

Results

- Plazomicin ($MIC_{50/90}$, 0.5/1 mg/L) inhibited 96.3% and 98.2% of the *Enterobacteriaceae* at ≤2 mg/L and ≤4 mg/L, respectively (Table 1)
  - Amikacin, gentamicin, and tobramycin inhibited 94.3%, 82.1%, and 75.9% of these isolates, respectively, when applying the current EUCAST breakpoints
- Plazomicin displayed similar activity against most *Enterobacteriaceae* species, including *E. coli* (n=1,182; $MIC_{50/90}$, 0.5/1 mg/L), *K. pneumoniae* (n=1,115; $MIC_{50/90}$, 0.25/0.5 mg/L), and *Enterobacter cloacae* (n=56; $MIC_{50/90}$, 0.25/0.5 mg/L; Table 1)
  - *Morganella morganii*, *Providencia*, and *Proteus* species displayed slightly higher plazomicin MIC_{50} (1-2 mg/L) and MIC_{90} (2-8 mg/L) values when compared to other *Enterobacteriaceae* species
- ESBLs were detected among 692 isolates displaying the CLSI ESBL-phenotypic criteria
  - The most common ESBL genes were $bla_{CTX-M-15}$ (n=486) and $bla_{CTX-M-14}$ (n=51; Figure 1)
  - Plazomicin ($MIC_{50/90}$, 0.25/1 mg/L) inhibited 94.7% and 94.8% of the 688 isolates carrying ESBL genes at ≤2 mg/L and ≤4 mg/L, respectively (Table 1)
  - Amikacin, gentamicin, and tobramycin inhibited 82.7%, 43.8%, and 25.0% of these isolates, respectively (Table 1)
- Plazomicin inhibited 78.8% of the 170 CRE at ≤2 mg/L or ≤4 mg/L (Table 1)
  - Amikacin and gentamicin inhibited 47.1% and 48.8% of the CRE isolates using EUCAST breakpoints
  - Tobramycin inhibited only 14.1% of the CRE isolates using EUCAST breakpoints
  - Carbapenemase genes were found in 138 (81.2%) CRE isolates and included 74 bla_{KPC}, 39 bla_{OXA-48}-like, and 26 bla_{NDM-1}
  - Plazomicin inhibited 60.6% of the 33 isolates carrying MBL genes at ≤0.25 mg/L
    - The remaining 13 MBL isolates had plazomicin MIC values at ≥128 mg/L
- AMEs were observed among 630/644 isolates tested and the most common genes were *aac(6')-Ib-cr* (n=353) and *aac(3)-IIa* (n=312; Figure 2)
  - Plazomicin ($MIC_{50/90}$, 0.5/2 mg/L) inhibited 92.7% and 93.5% of the AME-carrying isolates at ≤2 mg/L and ≤4 mg/L, respectively
  - Amikacin, gentamicin, and tobramycin inhibited 75.1%, 17.5%, and 1.7%, respectively, of these isolates using the EUCAST breakpoints
- Among 50 (1.5%) isolates displaying plazomicin MIC values >8 mg/L, 49 carried 16S rRNA methyltransferase genes
  - These isolates were highly resistant to all of the other aminoglycosides tested and displayed plazomicin MIC values of ≥128 mg/L
  - One *Providencia stuartii* from Germany carrying *aac(2')-Ia* displayed plazomicin MIC results of >128 mg/L
- The activity of plazomicin was similar in all 3 geographic regions (Figure 2)

Table 1 Activity of plazomicin and clinically available aminoglycosides

Organism/organism group	Plazomicin (mg/L)		% of isolates inhibited by plazomicin at (mg/L)					% susceptible using EUCAST breakpoint		
	MIC_{50}	MIC_{90}	0.5	1	2	4	8	Amikacin	Gentamicin	Tobramycin
Enterobacteriaceae (3,238)	0.5	1	74.1	90.8	96.3	98.2	98.5	94.3	82.1	75.9
Escherichia coli (1,182)	0.5	1	65.1	95.3	99.7	100.0		97.0	82.7	78.6
Klebsiella pneumoniae (1,115)	0.25	0.5	94.8	96.0	96.3	96.4	96.4	88.3	74.3	62.7
Klebsiella oxytoca (182)	0.5	0.5	94.0	100.0				100.0	98.3	97.3
Enterobacter cloacae species complex (120)	0.25	0.5	93.3	95.0	95.0	96.7	96.7	94.2	87.5	82.5
Enterobacter aerogenes (97)	0.5	0.5	90.7	97.9	97.9	97.9	97.9	97.9	95.9	95.9
Serratia marcescens (121)	1	1	40.5	94.2	100.0			95.9	95.0	78.5
Citrobacter freundii species complex (50)	0.5	0.5	98.0	100.0				100.0	92.0	86.0
Citrobacter koseri (82)	0.25	0.5	92.7	98.8	98.8	98.8	98.8	98.8	98.8	98.8
Morganella morganii (93)	2	4	16.1	46.2	81.7	94.6	100.0	98.9	86.0	90.3
Providencia spp. (39)	2	8	7.7	46.2	71.8	87.2	94.9	94.7	66.7	69.2
Proteus mirabilis (116)	2	4	0.9	14.7	68.1	98.3	99.1	99.1	75.9	78.4
Proteus vulgaris group (41)	1	2	24.4	70.7	100.0			100.0	100.0	100.0
CRE (170)	0.25	>128	71.8	78.2	78.8	78.8	78.8	47.1	48.8	14.1
Carbapenemase-producers (138)	0.25	>128	70.3	76.8	77.5	77.5	77.5	43.5	50.0	11.6
ESBL-producers (692)	0.25	1	79.0	92.2	94.7	94.8	94.8	82.7	43.8	25.0
AME-producers (630)	0.5	2	73.5	88.1	92.7	93.5	93.5	75.1	17.5	1.7
16S RNA methylase-producers (48)	>128	>128	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Figure 1 Distribution of genes encoding carbapenemases, 16S rRNA methyltransferases, AMEs, and ESBLs

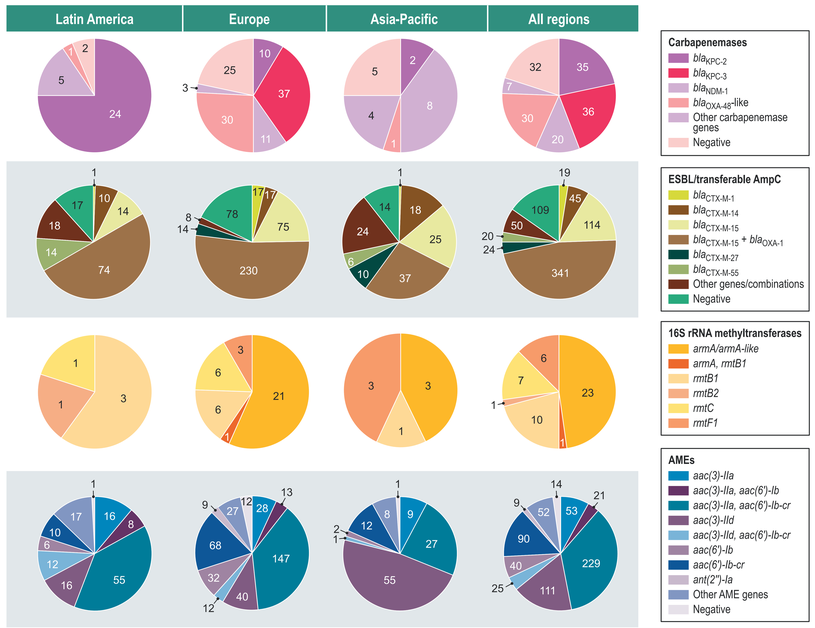
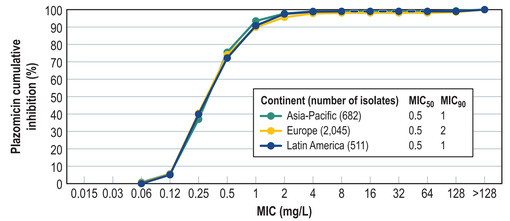


Figure 2 Activity of plazomicin by geographic region



Conclusions

- Plazomicin displayed activity against *Enterobacteriaceae* isolates collected on 3 continents
  - This collection included isolates with genes encoding ESBLs, carbapenemases, and AMEs
    - Plazomicin was the most active aminoglycoside against these groups of isolates
- Plazomicin is currently under review at the FDA for approval to treat complicated urinary tract infections, including acute pyelonephritis and bloodstream infections due to certain *Enterobacteriaceae* in patients who have limited or no alternative treatment options

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