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Plazomicin Activity against European *Enterobacteriaceae* Isolates Carrying Aminoglycoside-Modifying Enzymes and 16S rRNA Methylases

M CASTANHEIRA[1], TB DOYLE[1], LN WOOSLEY[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

Amended Abstract

Background: Production of aminoglycoside-modifying enzymes (AME) is the most frequent aminoglycoside resistance mechanism in *Enterobacteriaceae*. Although 16S rRNA methylases (RNAmet) are not as common, they confer high-level resistance against virtually all aminoglycosides. We evaluated the occurrence of AME and RNAmet among aminoglycoside-nonsusceptible *Enterobacteriaceae* isolates collected in 26 European countries during 2014-2015 and the activity of plazomicin (PLZ) and comparators against these isolates.

Materials/methods: *Enterobacteriaceae* isolates (n=4,217) from 45 European hospitals were susceptibility tested using the reference broth microdilution method. Isolates displaying nonsusceptible MICs (CLSI criteria) for gentamicin (GEN), amikacin (AMK), and/or tobramycin (TOB) were screened for *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 mg/L were tested for RNAmet.

Results: Among 783 (18.6% overall) isolates tested for AMEs, 583 carried *aac(6')-Ib*, 446 carried *aac(3)-IIa*, 40 carried *ant(2")-Ia*, and 20 isolates carried 4 other genes: *aac(3)-IVa*, *aac(3)-Ia*, *aac(3)-Id/e*, and *aph(3')-VIa*. Combinations of these genes were detected among 348 isolates and the most common was *aac(6')-Ib-aac(3)-IIa* (318 isolates). Only 56 (7.2%) isolates were negative for AME genes tested. AME producers were more common in Poland (73.5% of collected isolates), Ukraine (62.5%), Russia (42.4%), Romania (37.5%), and Turkey (29.5%). AME genes were mainly detected among *Klebsiella pneumoniae* (469/503) and *Escherichia coli* (216/233), but also in 5 other species. RNAmet were detected among 59 of 60, 1.4% overall, isolates displaying resistance to all aminoglycoside and PLZ MIC values of ≥128 mg/L (23 *rmtB*, 16 *armA* [1 also carrying *rmtA*], 12 *rmtC*, 8 *rmtF*). PLZ inhibited 720/727 (99.0%) isolates harbouring AME genes at ≤2 mg/L and 21/81 isolates nonsusceptible to all aminoglycosides displayed PLZ MICs ranging from 0.25 mg/L to 4 mg/L. Among the 7 AME producers displaying PLZ MIC values of 4-16 mg/L, 3 *K. pneumoniae*, 2 *E. coli*, and 2 *Proteus mirabilis* were noted. Only 2 isolates were AMK-nonsusceptible and displayed PLZ MICs of 0.5 mg/L and 4 mg/L. RNAmet producers displayed high MICs to all aminoglycosides.

Conclusions: PLZ showed stability against common AME genes detected in European countries and was more active than AMK, GEN, or TOB against AME-producing isolates. RNAmet producers were uncommon and resistant to all aminoglycosides. These data support the current development plan for plazomicin to treat serious infections caused by resistant *Enterobacteriaceae* where treatment options are limited.

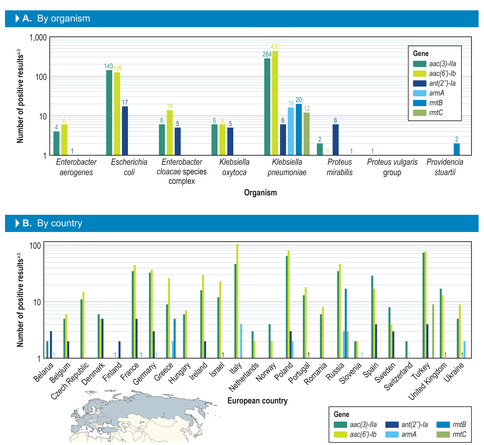
Introduction

- The most common resistance mechanisms to aminoglycoside agents in gram-positive and -negative bacteria are enzymatic modification and inactivation of aminoglycosides mediated by aminoglycoside-modifying enzymes (AME)
- AMEs encode elevated MIC levels to different aminoglycoside molecules by catalyzing the covalent modification of amino or hydroxyl groups in the aminoglycoside molecule, generating a product that binds poorly to the ribosome
- AMEs are grouped according to the type of modification that they catalyze and are aminoglycoside acetyltransferases (AACs), nucleotidyltransferases or adenyltransferases (ANTs), and phosphotransferases (APHs)
- Plazomicin is a semi-synthetic aminoglycoside derived from sisomicin and contains structural modifications that allow it to retain activity in the presence of AMEs
- Plazomicin is very active against *Enterobacteriaceae*, some *Pseudomonas aeruginosa*, and *Staphylococcus* spp., including methicillin-resistant (MRSA) isolates
- Consistent with other aminoglycosides, plazomicin is affected by ribosomal modifications by 16S rRNA methylases that are less common than AMEs and encode for high levels of resistance to virtually all aminoglycosides
- In this study, we evaluated the occurrence of AMEs and 16S rRNA methylases among aminoglycoside-nonsusceptible *Enterobacteriaceae* isolates collected in 26 European countries during 2014-2015 and the activity of plazomicin and comparators against these isolates

Materials and Methods

- A total of 4,217 *Enterobacteriaceae* bacterial isolates that are deemed cause of infection were limited to 1 per patient episode were included in the study
- Isolates collected during 2014-2015 from 26 European 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
- *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 mg/L 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 generating amplification in a multiplex reaction for AME genes were re-amplified in a singleplex reaction for confirmation
- *Enterobacteriaceae* isolates displaying plazomicin MIC results at ≥128 mg/L were screened for the presence of 16S rRNA methylases encoding genes *rmtA-H*, *armA*, and *npmA*
  - Amplicons for 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/)

Figure 1 Distribution of aminoglycoside resistance genes among aminoglycoside-nonsusceptible *Enterobacteriaceae* isolates from European hospitals by bacterial species and country



[1]Isolates might carry multiple AME genes
[2]Isolates were also positive for *aac(3)-Ia*, *aac(3)-Id/e*, *aac(3)-IVa*, *aph(3')-VIa*, *rmtA*, and *rmtF*

Results

- A total of 783 (18.6% overall) isolates displayed nonsusceptible MIC results for 1 to 3 of the clinically available aminoglycosides and were tested for AME-encoding genes: 503 *K. pneumoniae*, 233 *E. coli*, and 47 isolates belonging to 5 other bacterial species
- Among 727 (92.8% of tested) isolates carrying AME genes, the most prevalent gene was *aac(6')-Ib* (583 isolates), followed by *aac(3)-IIa* (446), *ant(2")-Ia* (40), *aac(3)-IVa* (8), *aph(3')-VIa* (6), *aac(3)-Ia* (5), and *aac(3)-Id/e* (1; Figure 1a)
- Combinations of AME-encoding genes were noted among 348 (44.4%) isolates and the most common was *aac(6')-Ib* plus *aac(3)-IIa* (318 isolates; data not shown)
- Bacterial species carrying AME genes were *K. pneumoniae* (469/503 tested isolates), *E. coli* (216/233), *Enterobacter aerogenes* (7/7), *E. cloacae* species complex (16/17), *K. oxytoca* (8/8), *P. mirabilis* (10/11), and *P. vulgaris* group (1/4)
- AME-producers were more common in Poland (73.6% of isolates tested), Ukraine (62.5%), Russia (42.4%), Romania (37.5%), and Turkey (29.5%; Figure 2)
- Among 783 isolates tested, 56 isolates (7.2%) were negative for AME genes
- Genes encoding 16S rRNA methylases were detected among 59 of 60 isolates (1.4% overall) displaying resistance to all aminoglycosides
- 16S rRNA methylase genes detected were *rmtB* (23 isolates), *armA* (16; 1 isolate also carrying *rmtA*), *rmtC* (12), and *rmtF* (8; Figure 1a)
- Isolates producing 16S rRNA methylase were detected among 56/57 *K. pneumoniae*, 1 *P. mirabilis*, and 2 *P. stuartii* tested, and these genes were observed in 11 European countries with 1 to 26 isolates detected (Figure 1b)
- Plazomicin (MIC_{50} range, 0.25-0.5 mg/L and MIC_{90} range, 1-4 mg/L) exhibited similar activity (±2-fold) against isolates nonsusceptible to gentamicin only, tobramycin only, amikacin and tobramycin, or gentamicin and tobramycin (Table 1)
- Isolates nonsusceptible to amikacin and gentamicin were not observed, and 2 isolates only nonsusceptible to amikacin displayed plazomicin MIC values at 0.5 mg/L and 4 mg/L
- Plazomicin inhibited 20/81 (24.7%) of the isolates displaying resistance to all tested aminoglycosides (all carried AMEs) at ≤2 mg/L, and 60/61 remaining isolates exhibited plazomicin MIC values at ≥128 mg/L (59 carried 16S rRNA methylase genes; Table 1)
- Plazomicin inhibited 99.0% of the isolates producing AMEs at ≤2 mg/L, including 99.1% of the isolates carrying *aac(6')-Ib*, 97.6% of the isolates harbouring *aac(3)-IIa*, 95.0% of the isolates carrying *ant(2")-Ia*, and 100.0% of the isolates carrying other AME genes (Table 1)
- The activity of other antimicrobial classes was variable among aminoglycoside-nonsusceptible isolates and isolates carrying AME genes (Table 2)
- Comparator agents, including colistin and tigecycline, displayed limited activity against all or some isolates producing 16S rRNA methylases (Table 2)

Figure 2 Occurrence of isolates carrying AME-encoding genes among aminoglycoside-nonsusceptible *Enterobacteriaceae* isolates from European hospitals

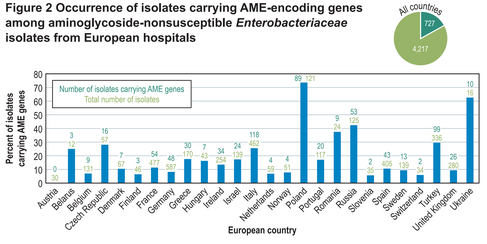


Table 1 Plazomicin activity against phenotypic and genotypic groups of *Enterobacteriaceae* isolates collected in Europe during 2014-2015

	No. of isolates at plazomicin MIC (mg/L; cumulative %)													
Organism groups[a]	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128	MIC_{50}	MIC_{90}
All *Enterobacteriaceae* (4,217)	14 0.3	247 6.2	1,302 37.1	1,441 71.2	769 89.5	267 95.8	93 98.0	16 98.4	6 98.6	0 98.6	1 98.6	19 99.0	41 100.0	0.5	2
Gentamicin-nonsusceptible only (74)	1 1.4	4 6.8	7 16.2	31 58.1	9 70.3	12 86.5	6 94.6	4 100.0						0.5	4
Tobramycin-nonsusceptible only (203)	0 0.0	29 14.3	84 55.7	53 81.8	28 95.6	4 97.5	4 99.5	1 100.0						0.25	1
Amikacin-nonsusceptible only (2)				0 0.0	1 50.0	0 50.0	1 100.0							1	
Gentamicin- and tobramycin-nonsusceptible only (474)	2 0.4	43 9.5	211 54.0	123 80.0	65 93.7	16 97.0	6 98.3	3 98.9	5 100.0					0.25	1
Amikacin- and tobramycin-nonsusceptible only (68)	0 0.0	3 4.4	29 47.1	19 75.0	14 95.6	3 100.0								0.5	1
Amikacin-, gentamicin-, and tobramycin-nonsusceptible (81)		0 0.0	10 12.3	5 18.5	4 23.5	1 24.7	1 25.9	0 25.9	0 25.9	0 25.9	0 25.9	19 49.4	41 100.0	>128	>128
All AME-producers[b] (727)	3 0.4	71 10.2	309 52.7	213 82.0	99 95.6	25 99.0	2 99.3	1 99.5	4 99.8					0.25	1
aac(6')-Ib (583)	2 0.3	65 11.5	279 59.3	159 86.6	65 97.8	9 99.3	0 99.3	1 99.5	3 100.0					0.25	1
aac(3)-IIa (446)	3 0.7	41 9.9	196 53.8	128 82.5	58 95.5	15 98.9	0 98.9	1 99.1	4 100.0					0.25	1
ant(2")-Ia (40)	0 0.0	2 5.0	6 20.0	22 75.0	4 85.0	4 95.0	2 100.0							0.5	2
aac(3)-IVa (8)			0 0.0	4 50.0	3 87.5	1 100.0								0.5	
aph(3')-VIa (6)	0 0.0	1 16.7	4 83.3	0 83.3	1 100.0									0.25	
aac(3)-Ia (5)		0 0.0	2 40.0	1 60.0	1 80.0	1 100.0								0.5	
aac(3)-Id/e (1)			0 0.0	1 100.0											
16S rRNA methylases positive (59)											0 0.0	19 32.2	40 100.0	>128	>128

[a]Nonsusceptible groups were defined applying CLSI breakpoint criteria. No amikacin-nonsusceptible only or gentamicin-nonsusceptible only isolates were observed
[b]Isolates might carry multiple AME genes

Table 2 Activity of plazomicin and comparator agents tested against phenotypic and genotypic groups of *Enterobacteriaceae* isolates collected in Europe during 2014-2015

	MIC_{50}/MIC_{90} (mg/L)							
Organism Group (no. tested)	Plazomicin	Amikacin	Gentamicin	Tobramycin	Meropenem	Piperacillin-tazobactam	Colistin	Tigecycline
All *Enterobacteriaceae* (4,217)	0.5 / 2	2 / 4	0.5 / >8	0.5 / >8	0.03 / 0.12	2 / >64	≤0.5 / >8	0.25 / 1
Gentamicin-nonsusceptible (74)	0.5 / 4	1 / 2	>8 / >8	4 / 4	0.03 / 0.12	2 / >64	≤0.5 / >8	0.25 / 1
Tobramycin-nonsusceptible (203)	0.25 / 1	4 / 16	0.5 / 2	>8 / >8	0.03 / 4	16 / >64	≤0.5 / 4	0.25 / 1
Gentamicin- and tobramycin-nonsusceptible (474)	0.25 / 1	4 / 16	>8 / >8	>8 / >8	0.03 / 8	16 / >64	≤0.5 / >8	0.25 / 1
Amikacin- and tobramycin-nonsusceptible (68)	0.5 / 1	32 / >32	1 / 2	>8 / >8	>32 / >32	>64 / >64	≤0.5 / >8	0.5 / 1
Amikacin-, gentamicin-, and tobramycin-nonsusceptible (81)	>128 / >128	>32 / >32	>8 / >8	>8 / >8	0.03 / 32	32 / >64	≤0.5 / >8	0.25 / 1
All AME (727)[a]	0.25 / 1	4 / 32	>8 / >8	>8 / >8	0.03 / 0.12	2 / >64	≤0.5 / >8	0.25 / 1
aac(6')-Ib (583)	0.25 / 1	4 / 32	>8 / >8	>8 / >8	0.03 / >32	64 / >64	≤0.5 / >8	0.25 / 1
aac(3)-IIa (446)	0.25 / 1	4 / 16	>8 / >8	>8 / >8	0.03 / 16	16 / >64	≤0.5 / >8	0.25 / 1
ant(2")-Ia (40)	0.5 / 2	2 / 8	>8 / >8	>8 / >8	0.03 / 0.12	8 / >64	≤0.5 / >8	0.25 / 4
aac(3)-IVa (8)	0.5 /	2 /	>8 /	>8 /	≤0.015 /	2 /	≤0.5 /	0.12 /
aph(3')-VIa (6)	0.25 /	>32 /	1 /	>8 /	16 /	>64 /	≤0.5 /	0.25 /
aac(3)-Ia (5)	0.5 /	4 /	8 /	8 /	0.06 /	16 /	≤0.5 /	0.25 /
16S rRNA methylases (59)	>128 / >128	>32 / >32	>8 / >8	>8 / >8	8 / >32	>64 / >64	≤0.5 / >8	0.5 / 2

[a]Isolates might carry multiple AME genes

Conclusions

- AMEs were detected in 727/783 tested isolates, mainly in *K. pneumoniae* and *E. coli*, from virtually all European countries surveyed
- The most common AME-encoding genes detected were *aac(6')-Ib* and *aac(3)-IIa* alone or in combination
- Genes encoding 16S rRNA methylases were detected in 59/60 isolates tested, and *rmtB* and *armA* were the most common genes
- Plazomicin was active against isolates nonsusceptible to gentamicin and/or tobramycin, amikacin plus tobramycin, and isolates producing AMEs
- Plazomicin at ≤2 mg/L inhibited approximately 25% of the isolates nonsusceptible to all 3 clinically available aminoglycosides tested; the remainder displayed elevated plazomicin MIC values and the majority of these (59/60) carried a gene encoding a 16S rRNA methylase
- Broad dissemination of AMEs in European countries and the activity of plazomicin against the isolates producing these enzymes warrants further development of this compound

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References

1. Clinical and Laboratory Standards Institute (2017). *M100-S27. Performance standards for antimicrobial susceptibility testing: 27th informational supplement*. Wayne, PA: CLSI.
2. 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.
3. 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.
4. Ramirez MS, Tolmasky ME (2010). Aminoglycoside modifying enzymes. *Drug Resist Updat* 13: 151-171.
5. Walkty A, Adam H, Baxter M, et al. (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.