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Impact of Changes in Growth Medium, Inoculum Density, and Incubation Conditions on the *In Vitro* Antimicrobial Activity of Plazomicin

LR DUNCAN[1], PR RHOMBERG[1], BA SCHAEFER[1], RK FLAMM[1], AW SERIO[2], KM KRAUSE[2], M CASTANHEIRA[1]

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

Contact Information:
Leonard R. Duncan, Ph.D.
JMI Laboratories
345 Beaver Kreek Centre, Suite A
North Liberty, IA 52317
Phone: (319) 665-3370
Fax: (319) 665-3371
Email: leonard-duncan@jmilabs.com

Abstract

Background: Plazomicin (PLZ) is a next-generation aminoglycoside (AMG) that overcomes common resistance mechanisms and has completed Phase 3 studies in patients with serious bacterial infections due to multidrug-resistant *Enterobacteriaceae*, including extended-spectrum β-lactamase-producing and carbapenem-resistant isolates. We evaluated how altering the reference broth microdilution (BMD) MIC methodology affected plazomicin's *in vitro* activity.

Methods: Using CLSI BMD methods, MIC values were measured in triplicate for 5 ATCC reference strains and 5 US clinical isolates against PLZ and amikacin (AMK; control compound) and compared to MIC values obtained using the following modified conditions: (1) inocula with 5×10^4 and 5×10^6 CFU/mL; (2) pH of 5.0, 6.0, and 8.0; (3) 10% human serum (HS), 50% HS, and 3.75% lysed horse blood (LHB); (4) nonstandard concentrations of Ca^{2+} and Mg^{2+}; and (5) an anaerobic or 5% CO_2 atmosphere. Modal (or median) MIC values were compared between each standard and modified test condition, and MIC shifts of >2-fold were considered significant.

Results: The conditions that significantly affected PLZ and AMK MIC values are shown below (Table). No significant changes in MIC values were observed for any of the strains when the inoculum density was lowered or when the broth was supplemented with LHB or 10% HS. Only 3 isolates had increased MIC values after adding 50% HS, and MIC values for only 1 strain were affected by altered cation concentrations. For both AMGs, acidic pH and anaerobic conditions significantly decreased activity against all and most isolates/strains tested, respectively.

Conclusions: Most tested parameters had little or no effect on PLZ or AMK MIC values; however, acidic and anaerobic conditions significantly decreased the activity of both antimicrobials. These results are similar to data previously reported for AMK and other members of the AMG class of antimicrobials.

Table Nonstandard parameters that affected plazomicin and amikacin MIC values by >2-fold relative to reference MIC conditions

	Inoculum (CFU/mL)	pH			Serum	$[Mg^{2+}]/[Ca^{2+}]$	Atmosphere	
Species (no.)	5×10^6	5	6	8	50% Serum	<5 mg/L for both cations[c]	Anaerobic	5% CO_2
S. aureus (2)	P(2), A(2)[a]	**P(2), A(2)**[b]	**P(2)**, A(2)	P(1)↓[c]	P(1), A(1)		**P(2), A(2)**	P(2), A(2)
E. coli (2)	P(1), A(1)	**P(2), A(2)**	P(2), A(2)	P(1)↓			**P(1), A(1)**	A(1)
P. aeruginosa (1)		**P(1), A(1)**	P(1), A(1)		P(1)	P(1)↓, A(1)↓		
K. pneumoniae (3)		**P(3)**, A(3)	P(3), A(3)				P(2), A(1)	
Enterobacter spp. (2)		**P(2), A(2)**	**P(2), A(2)**		A(1)		P(2), A(2)	

[a]P, plazomicin; A, amikacin; (2), (no. isolates affected); empty cell, change in MIC value was ≤2-fold for both antimicrobials
[b]Bold data points, MIC values increased by ≥8-fold for all indicated strains/isolates
[c]↓ symbol indicates that MIC values decreased under non-reference conditions (all other affected MIC values increased)
[d]No other combination of Mg^{2+}/Ca^{2+} concentrations affected MIC values by more than 2-fold for any isolate/strain

Introduction

- Plazomicin (PLZ) is a semi-synthetic aminoglycoside derived from sisomicin that contains structural modifications that allow it to retain antibacterial activity in the presence of aminoglycoside-modifying enzymes
- PLZ is being developed to treat serious bacterial infections due to multidrug-resistant *Enterobacteriaceae* (ENT), including carbapenem-resistant ENT (CRE), and has shown positive results in 2 Phase 3 clinical trials
- The *in vitro* activity of some antimicrobials can be affected by small variations in interlaboratory testing conditions, and a drug's *in vivo* antimicrobial activity can be affected by conditions (eg, protein binding) that are not replicated using standard *in vitro* MIC testing conditions
- For these reasons, both the US Food and Drug Administration and the Clinical and Laboratory Standards Institute recommend investigating the impact of nonstandard MIC testing conditions (eg, the presence of human serum or nonstandard pH) on the *in vitro* antimicrobial activity of novel agents
- Here we report on how variations of the standard *in vitro* testing parameters affected the antimicrobial activity of PLZ

Materials and Methods

- MIC values for PLZ and amikacin (AMK; control compound) were measured in triplicate under standard and nonstandard conditions (tested ranges: 0.06–64 µg/mL)
  - Standard MIC testing conditions
    - CLSI broth microdilution methodology was used
    - Isolates were tested in Mueller Hinton broth (pH 7.2–7.4) supplemented with 20–25 mg/L of Ca^{2+} and 10–12.5 mg/L of Mg^{2+} (CA-MHB)
    - Panels were inoculated with 5×10^5 colony-forming units (CFU) per mL, incubated at 35°C in ambient air, and read after 16–20 hours of incubation
  - Nonstandard MIC testing conditions
    - **Inoculum size:** MIC values were measured using inocula that contained 5×10^4 (1/10th standard) and 5×10^6 (10X standard) CFU/mL
    - **Broth pH:** Isolates were tested in CA-MHB adjusted to pH values of 5.0, 6.0, and 8.0
    - **Presence of human serum and lysed horse blood (LHB):** Isolates were tested in CA-MHB that contained 10% (v/v) pooled, heat-inactivated human serum (HS), 50% (v/v) HS, and 3.75% (v/v) lysed horse blood (LHB)
    - **Altered Mg^{2+} and Ca^{2+} concentrations:** All 10 strains were tested in a 3 × 3 MIC matrix using MHB supplemented with Ca^{2+} and Mg^{2+} at concentrations below (<5 mg/L), at, and above (Ca^{2+}, 50 mg/L; Mg^{2+}, 25 mg/L) the concentrations used under standard conditions; thus, 90 independent MIC values were generated for each antimicrobial
    - **Atmosphere:** MIC values were measured using panels that were incubated under anaerobic conditions (AnaeroPack; Mitsubishi Gas Chemical) and in 5% CO_2
- Quality control (QC) was ensured per CLSI M07-A10 and M100-S27 using 3 reference strains: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853
  - Inocula concentrations were monitored to ensure the prescribed number of bacterial cells was present for MIC testing
- 10 bacterial strains from 6 different species were tested (Table 1)
  - Tested strains included 5 ATCC reference strains and 5 clinical isolates (collected in 2015)
  - CLSI interpretive criteria were used
- Data reporting
  - In total, 380 different combinations of strain, drug, and MIC condition were evaluated
  - In 378/380 instances, the MIC values agreed within 2-fold for each triplicate data set, and the modal value was reported
  - In 2/380 instances, the triplicate data points agreed within 4-fold and the median value was reported
  - A 4-fold difference in MIC values between standard and nonstandard testing conditions was considered to be significant

Results

- Table 1 displays the PLZ and AMK MIC values for all tested strains and clinical isolates measured under standard conditions
- Where available, all PLZ and AMK MIC values for ATCC reference strains were within established QC ranges (Table 1 and data not shown)
- When an inoculum that contained 10-fold more CFU/mL than standard conditions was used, the MIC values for both PLZ and AMK were increased 4-fold for the 2 *S. aureus* strains and *E. coli* ATCC 25922 (Figure 1)
  - No other strain/antimicrobial combination MIC value was affected by more than 2-fold
- All MIC values for PLZ and AMK using CA-MHB at pH 5 or 6 were significantly higher than the MIC values obtained under standard conditions (Figure 2)
  - In some cases, MIC values were increased by >100-fold
  - MIC values at pH 8 were similar to standard conditions except for 2 strains, where the plazomicin MIC values were 4-fold lower than those measured at pH 7 (Figure 2B)
- The presence of 50% human serum (HS) increased the AMK and PLZ MIC values by 4-fold for 2 strains each, while 10% HS and 3.75% lysed horse blood did not affect any MIC values by more than 2-fold (Figure 3)
- Only 2 instances (of 160 MIC values) occurred in which nonstandard cation concentrations impacted the aminoglycoside MIC values compared to standard conditions: when Mg^{2+} and Ca^{2+} were both present at concentrations below standard levels (<5 mg/L), the *P. aeruginosa* ATCC 27853 AMK and PLZ MIC values were each decreased by 4-fold (data not shown)
- Under anaerobic conditions, the potencies of AMK and PLZ were decreased by ≥4-fold against most of the tested strains (Figure 4)
- An atmosphere containing 5% CO_2 also increased the MIC values of 3 (AMK) and 2 (PLZ) tested strains

Conclusions

- The following nonstandard *in vitro* MIC testing parameters had little or no effect on PLZ or AMK MIC values for the majority of tested isolates
  - Increased or decreased inoculum cell density
  - Addition of human serum or lysed horse blood
  - Altered levels of Mg^{2+} and Ca^{2+}
  - Growth in 5% CO_2 or at pH 8
- In contrast, acidic pH conditions significantly decreased the potency of both aminoglycosides for all 10 isolates
- The majority of isolates also exhibited increased PLZ and AMK MIC values when grown anaerobically
- Decreased activity of aminoglycosides under acidic or anaerobic conditions is a well-documented phenomenon
- Thus, only a few nonstandard MIC conditions affected PLZ MIC values, and AMK behaved similarly

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Table 1 Strains and isolates tested and MIC values under standard conditions

Species/strain name	Abbreviated name	Origin	Relevant phenotype	Modal MIC (µg/mL)	
				AMK	PLZ
Staphylococcus aureus					
ATCC 29213	SA1	reference strain	MSSA, AMK-S	1	0.5
C#884592	SA2	clinical isolate	MRSA, AMK-S	2	0.25
Escherichia coli					
ATCC 25922	EC1	reference strain	non-ESBL, AMK-S	1	0.5
C#880555	EC2	clinical isolate	non-ESBL, AMK-S	16	8
Klebsiella pneumoniae					
ATCC 700603	KPN1	reference strain	ESBL, AMK-S	0.5	0.25
ATCC BAA-1705	KPN2	reference strain	CRE, AMK-I	32	0.25
C#887911	KPN3	clinical isolate	CRE, AMK-S	4	1
Enterobacter aerogenes					
C#877253	EAE	clinical isolate	non-ESBL, AMK-S	1	0.5
Enterobacter cloacae					
C#882281	ECL	clinical isolate	ESBL, AMK-S	0.5	0.25
Pseudomonas aeruginosa					
ATCC 27853	PSA	reference strain	AMK-S	2	2

C#, JMI collection number; MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; ESBL, extended-spectrum β-lactamase; CRE, carbapenem-resistant *Enterobacteriaceae*; AMK-S, amikacin-susceptible; AMK-I, amikacin intermediate; PLZ, plazomicin

Figure 1 Effect of inoculum size on MIC values

See Table 1 for strain details. Red asterisks denote MIC values that are 4-fold higher than the MIC value under standard conditions.

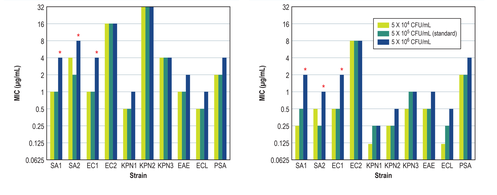


Figure 2 Effect of broth medium pH on MIC values

See Table 1 for strain details. All pH 5 and 6 MIC values were significantly higher than the standard condition. Red asterisks denote MIC values that are 4-fold lower than the MIC value under standard conditions. MIC values of >64 were plotted as 128 µg/mL.

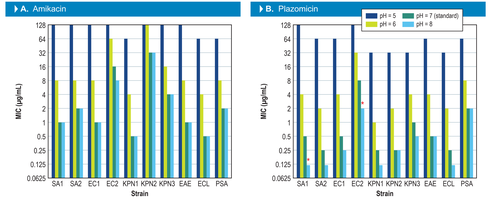


Figure 3 Effect of human serum (HS) and lysed horse blood (LHB) on MIC values

See Table 1 for strain details. Red asterisks denote MIC values that are 4-fold higher than the MIC value under standard conditions.

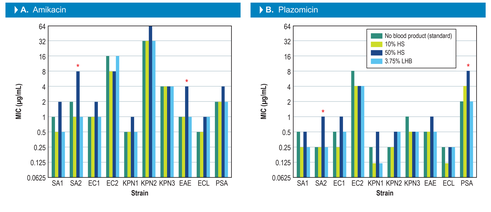
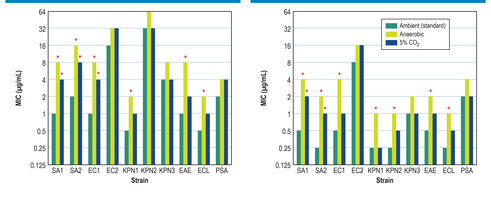


Figure 4 Effect of different growth atmospheres on MIC values

See Table 1 for strain details. Red asterisks denote MIC values that are ≥4-fold larger than the MIC value under standard conditions.



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