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Minocycline Activity is Enhanced by Polymyxin B in *tetB*-containing Isolates of *Acinetobacter baumannii*

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Revised Abstract

Background: MINO has recently emerged as one of the few treatment options for infections caused by multidrug resistant strains of ACB. Acquired MINO resistance (R) in ACB is associated with the presence of the *tetB* efflux pump. The objective of this study was to assess activity of MINO in combination with PmB against the *tetB*-containing strains of ACB, including PmB-R strains.

Methods: The in vitro activity of MINO in combination with PmB was evaluated by checkerboard assays against the panel of 167 *tetB*-containing clinical isolates of *Acinetobacter baumannii*, including four PmB-resistant isolates. The presence of the *tetB* gene was confirmed by PCR. The pmrCABoperon was sequenced in PmB-R strains. 93% and 100% of strains were also R to meropenem and levofloxacin, respectively.

Results: MINO and PmB MIC50/MIC90 (range) were 16/32 (0.5-32) µg/ml and 1/2 (0.5-8) µg/ml, respectively. Only 12% of *tetB*-containing isolates were susceptible (S) to ≤ 4 µg/ml MINO (FDA & CLSI susc. breakpoint). PmB at 0.25 or 0.5 µg/ml increased MINO potency 2 -256-fold bringing MIC50/MIC90 to 0.5/4 µg/ml. 100% of *tetB* strains were S to MINO with PmB at 0.5 µg/ml (Table). Four PmB-R strains (PmB MIC ≥ 4 µg/ml), had mutations in pmrB. In these strains, the MINO MIC alone or in combination with PmB at 0.5 µg/ml ranged from 8 to 32 µg/ml and from 2 to 4 µg/ml, respectively.

Conclusion: PmB enhanced the potency of MINO against *tetB*-containing ACB isolates, including those resistant to PmB. This combination warrants further evaluation in the management of patients with infections due to ACB.

Introduction

Minocycline is FDA-approved for the treatment of infections due to *Acinetobacter* spp. A new IV formulation of minocycline (Minocin) was approved by the US FDA in 2015. It remains one of the most active agents in vitro against multidrug resistant isolates of *Acinetobacter baumannii.*

Increasing resistance to polymyxin B has further limited the options for treatment of Acinetobacter spp.

Among *A. baumannii* isolates collected globally from 2010 to 2014, the rate of minocycline resistance (6.6%) was lower than that of any other antibacterial agent tested (30.9% to 50.3%) [1].

Minocycline is a poor substrate of the multi-drug transporter *adeABC* in Acinetobacter. The major mechanisms of resistance to minocycline is mediated by the TetB transporter; absence of *tetB* is a good indicator of minocycline susceptibility in ACM. (Lomovskaya, et al; ID Week 2016; Poster 2043).

The objective of this study was to assess activity of minocycline in combination with PmB against the *tetB*-containing strains of ACB, including PmB-R strains.

Methods

Organism collection

tetB-containing clinical isolates of *Acinetobacter baumannii* (N=167) were selected for susceptibility testing, including four PmB-resistant isolates (PmB MIC ≥ 4 µg/ml). The presence of the *tetB* gene was confirmed by PCR. 93% and 100% of strains were also resistant to meropenem and levofloxacin, respectively.

MICs of *tetB*-containing Strains of *A. baumannii*

	Minocycline	Polymyxin B	Levofloxacin	Meropenem
MIC_{50}	16	1	32	64
MIC_{90}	32	2	>64	>64

Susceptibility testing

MICs were determined using Clinical and Laboratory Standards Institute (CLSI) broth microdilution reference methods (M07-A10; 2015 [2]). Combinations of antibiotics were tested in a checkerboard format in a 96-well panel according to the Moody procedures in Clinical Microbiology Procedures Handbook [2,4].

MBC assays were conducted at ~10^5 and ~10^6 CFU/mL inoculum following M26-A guidelines [3].

Titrations were plated for all wells of select MBC plates with ~10^6 CFU/mL inoculum to confirm bactericidality.

Sequencing of resistance related genes

The presence of the *tetB* gene was confirmed by PCR

The *pmrCAB* operon was sequenced in PmB-R strains. ATCC 17978 and ATCC 19606 was used as templates/wild-type references for alignments [5,6,7,8].

Results

Table 1: Distribution of minocycline and polymyxin B MICs alone and in combination against 167 *tetB*-containing strains of *Acinetobacter baumannii*

MIC (µg/ml)	MINO alone	MINO w/ 0.125 µg/ml of PmB	MINO w/ 0.25 µg/ml of PmB	MINO w/ 0.5 µg/ml of PmB	PmB alone
≤0.06	0.0%	0.0%	0.0%	41.3%	0.0%
0.125	0.0%	0.0%	0.0%	47.3%	0.0%
0.25	0.0%	0.0%	0.0%	49.7%	0.0%
0.5	1.2%	1.2%	1.8%	57.5%	35.3%
1	1.8%	1.8%	3.0%	65.3%	88.6%
2	3.6%	4.2%	6.6%	79.6%	96.4%
4	12.0%	18.0%	58.1%	100.0%	98.8%
8	34.1%	77.2%	95.2%	100.0%	100.0%
16	86.2%	100.0%	100.0%	100.0%	100.0%
32	100.0%	100.0%	100.0%	100.0%	100.0%

MIC_{50} and MIC_{90} are in **red**
Dashed line indicates CLSI and FDA susceptibility breakpoint for minocycline

- Minocycline and polymyxin B MIC_{50}/MIC_{90} (range) were 16/32 (0.5-32) µg/ml and 1/2 (0.5-8) µg/ml, respectively.
- Only 12% of *tetB*-containing isolates were susceptible to ≤4 µg/ml minocycline (FDA & CLSI susceptibility breakpoint).
- Polymyxin B at 0.25 or 0.5 µg/ml increased minocycline potency 2 to 256-fold bringing the MIC_{50}/MIC_{90} to 0.5/4 µg/ml.
- 100% of *tetB*-containing strains were susceptible to minocycline when tested with PmB at 0.5 µg/ml.

Figure 1. Concentration-dependent effect of polymyxin B on minocycline MICs in *tetB*-containing *Acinetobacter baumannii* (N=167)

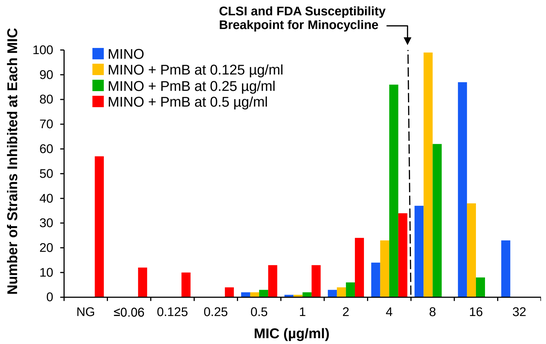


Table 2: Sensitivity to minocycline is increased by addition of polymyxin B, even in polymyxin and minocycline-resistant strains of *Acinetobacter baumannii*

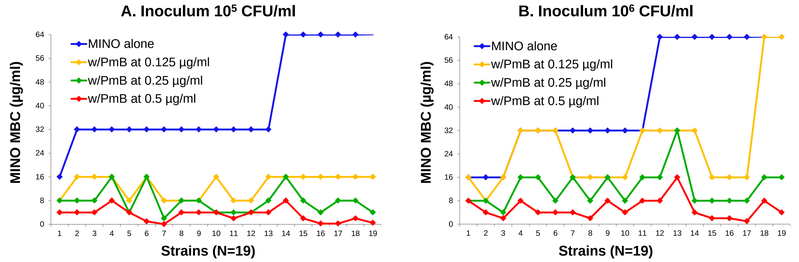
- Four *tetB*-carrying PmB-R strains (PmB MIC ≥ 4 µg/ml), had mutations in *pmrB*. In these strains, the minocycline MICs in combination with polymyxin B (0.5-2 µg/ml) were reduced from 8-32 µg/ml to 2 - 4 µg/ml.

		Polymyxin resistance gene mutation		MINO MIC (µg/ml) in the presence of PmB (µg/ml)								
Strain	*tetB* positive	*pmrA*	*pmrB*	0	0.125	0.25	0.5	1	2	4	8	PmB MIC
AB1211	yes	WT	A138T, P233A	32	8	8	4	4	4	4	2	4
AB1292	yes	WT	S14P	8	8	4	4	2	2	2	2	8
AB1312	yes	WT	A138T, A226V	8	8	4	4	2	2	2	ng	4
AB1327	yes	WT	A138T, A226V	8	4	4	2	2	2	ng	ng	4

NG=no growth

Figure 2. Effect of inoculum size on polymyxin B enhancement of minocycline MBC in *Acinetobacter baumannii*

- 19 strains with PmB MIC > 0.5 µg/ml were selected for MBC experiments with inoculum of 10^5 and 10^6 CFU/ml



- Minocycline alone and in combination with polymyxin B showed bactericidal activity against *A. baumannii.*
- Potentiation of bactericidal activity of minocycline with addition of polymyxin B was demonstrated in minocycline-resistant *A. baumannii* isolates.
- Potentiation of bactericidal activity was observed at both 10^5 CFU/ml and 10^6 CFU/ml starting inoculum.

Table 3: Titration of checkerboard wells using a representative *tetB*-containing strain

- The extent of killing by minocycline/PmB combinations was higher than that of each of these drugs alone

PmB concentration (µg/ml)	Minocycline concentration (µg/ml)											
	64	32	16	8	4	2	1	0.5	0.25	0.125	0.06	0
8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8
4	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8
2	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8
1	-4.8	-4.8	-4.8	-4.8	-4.8	-4.0	-4.8	-4.8	-4.8	-4.8	-4.8	-4.8
0.5	-4.8	-4.5	-4.8	-4.8	-4.8	1.6	2.3	1.5	1.8	1.6	1.7	1.6
0.25	-4.8	-4.8	-4.8	-0.2	2.2	2.4	2.4	2.4	2.3	2.3	2.4	2.4
0.125	-4.0	-2.2	-1.5	1.6	2.1	2.3	2.3	2.3	2.2	2.3	2.4	2.4
0	-1.8	-2.6	1.2	1.9	2.4	2.3	2.5	2.4	2.3	2.2	2.2	2.4

Values are $\Delta\log_{10}$ cfus relative to starting inoculum
Red text marks ≥3 logs of killing

Conclusions

- Polymyxin B enhanced the potency of minocycline against *tetB*-containing *Acinetobacter baumannii* isolates, including those resistant to polymyxin B and minocycline.
- Polymyxin B potentiated the MBCs of minocycline as well
- This combination of minocycline with polymyxin B warrants further evaluation in the management of patients with infections due to *Acinetobacter baumannii,* including isolates resistant to one or both drugs.

Disclaimers/Acknowledgements

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