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TetB Testing and its Absence Identifies Minocycline (MINO) Susceptible Isolates of *Acinetobacter baumannii* (ACB)

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Revised Abstract

Background: MINO is one of the few options available to treat infections caused by ACB. Acquired MINO resistance (R) in ACB is associated with the presence of the TetB efflux pump. Previous studies demonstrated that in the absence of *tetB*, other combinations of intrinsic mechanisms of resistance do not produce MINO MICs above the current susceptibility breakpoint (≤ 4 µg/ml). The objective of this study was to assess whether *tetB* testing can discriminate between MINO S and non-susceptible (non-S) isolates.

Methods: 258 ACB isolates collected from multiple geographic sources were tested for MINO susceptibility using CLSI broth microdilution reference methods and for the *tetB* gene by PCR. MIC frequency distributions for the *tetB* positive (POS) and negative (NEG) sets of isolates were generated and assessed for the degree of separation. Sensitivity, specificity and positive and negative predictive values (PPVs and NPVs, respectively) of the *tetB* PCR to predict minocycline susceptibility (MIC values of ≤4 µg/ml) and non-susceptibility (MIC values of >4 µg/ml) were determined.

Results: Of the 258 ACB, 165 and 93 isolates were found to be *tetB*-POS and NEG, respectively. MIC frequency distributions of *tetB*-POS and *tetB*-NEG sets demonstrated high degree of separation (Figure). Of the *tetB*-negative strains, all 93 (100%) were susceptible to MINO (MINO MIC ≤4 µg/ml, MINO S according to FDA SB), resulting in a negative predictive value (NPV) of 100%. Of 165 *tetB*-positive strains, 154 were non-susceptible to minocycline (MINO MIC >4 µg/ml) resulting in a positive predictive value (PPV) of 93.3%.

Conclusions: *tetB* testing of clinical isolates might constitute a potential rapid susceptibility test where the absence of *tetB* is predicts susceptibility to minocycline. Further studies are warranted.

Introduction

Minocycline, a semisynthetic tetracycline derivative, is FDA approved for the treatment of infections due to *Acinetobacter* spp. A new formulation of MINOCIN® (minocycline) for injection was approved by the US FDA in 2015.

Recent global surveillance studies show the susceptibility of *Acinetobacter* spp, to minocycline ranges from 72.3% to 84.5%, with MDR isolates ranging between 66.2% to 75.4%, respectively [1].

Acquired minocycline resistance in *Acinetobacter baumannii* is most frequently caused by the TetB efflux pump [2].

Previous studies demonstrated that in the absence of *tetB*, other combinations of intrinsic mechanisms of resistance do not produce minocycline MICs above the current susceptibility breakpoint (≤ 4 µg/ml) [3].

The objective of this study was to assess whether *tetB* testing can discriminate between minocycline susceptible and non-susceptible isolates of Acinetobacter.

Methods

Organism collection

258 clinical isolates of *Acinetobacter baumannii* (N=258) collected worldwide during 1998 to 2015 were selected for susceptibility testing and testing for the presence of the *tetB* gene.

Susceptibility testing

MICs were determined using Clinical and Laboratory Standards Institute (CLSI) broth microdilution method as described in CLSI document M07-A10 (2015) [4].

Detection of the *tetB* gene in the strains of *A. baumannii*

Detection of the *tetB* gene was carried out by PCR. Cells of a fresh single colony were resuspended in 100 µl of water, heated for 10 min at 95ºC and used as PCR template.

The primers for the *tetB* gene were *tetB*-F (5'- TACGTGAATTTATT GCTTCGG -3') and *tetB*-R (5'- ATACAGCATCCAA AGCGCAC -3'), located within the *tetB* coding region.

The full-length *tetB* gene was detected using primers representing the 5' and 3' ends of the *tetB* coding region in PCR. These primers were *tetB*-clone-F (5'- ACACGGATCCATAGAGAAAAGTGAAATG-3') and *tetB*-clone-R (5'-ACGCTCTAGAGATTTATTTGTGGAACTG ACA-3').

PCR reactions were performed using DreamTaq Green Master mix (ThermoFisher) under the following conditions: 95ºC for 5 min, followed by 35 cycles of 95ºC for 20 sec, 55ºC for 20 sec and 72ºC for 1 min, and finally 72ºC for 5 min.

The PCR products were analyzed on a 1% agarose gel. In some tests, the presence of *tetB* was also examined by quantitative PCR (qPCR) using the primers *tetB*-F and *tetB*-R.

The heated cells were properly diluted with water and 9 µl was mixed with 10 µl of SYBR® Select Master Mix (2x) (ThermoFisher) and 1 µl of the primer pair mix for final primer concentrations of 0.5 µM.

qPCR was run on an ABI 7000 Sequence Detection System with the following thermal cycling conditions: 50ºC for 2 min, 95ºC for 5 min, followed by 40 cycles of 95ºC for 15 sec and 60ºC for 1 min.

Statistical analysis

Sensitivity, specificity and positive and negative predictive values (PPVs and NPVs, respectively) of the *tetB* PCR to predict minocycline susceptibility (MIC values of ≤4 µg/ml) and non-susceptibility (MIC values of >4 µg/ml) were determined using the above phenotypically characterized clinical isolates of *A. baumannii*.

The antimicrobial susceptibility data were used as the reference results for these calculations, and comprised 93 isolates that were minocycline susceptible and 165 that were minocycline non-susceptible.

Results

Table 1. Antibiotic susceptibility pattern (MIC, µg/ml) of the strains of *A. baumannii* used in this study (n=258)

	Tigecycline	Minocycline	Doxycycline	Polymyxin B	Levofloxacin	Meropenem
MIC_{50}	4	8	64	1	16	32
MIC_{90}	8	32	>64	2	64	>64
Range	0.125-32	≤0.06-32	≤0.06->64	0.5-32	≤0.06->64	≤0.06->64
Susc.	No BP	40.3%	29.8%	No BP	3.3%	12.4
Intermediate	No BP	15.1%	1.6%	No BP	3.7%	7.0
Resistant	No BP	44.6%	68.6%	No BP	93%	80.6

- 258 *A. baumannii* isolates collected from multiple geographic sources were tested for minocycline susceptibility and for presence of the *tetB* gene by PCR.
- MIC frequency distributions for *tetB* positive (POS) and negative (NEG) sets of isolates were generated and assessed for the degree of separation.
- MIC frequency distributions of *tetB*-POS and *tetB*-NEG sets demonstrated a high degree of separation (Figure 1).

Figure 1. MIC frequency distributions for *tetB* positive and negative sets of isolates of *A. baumannii* (n=258)

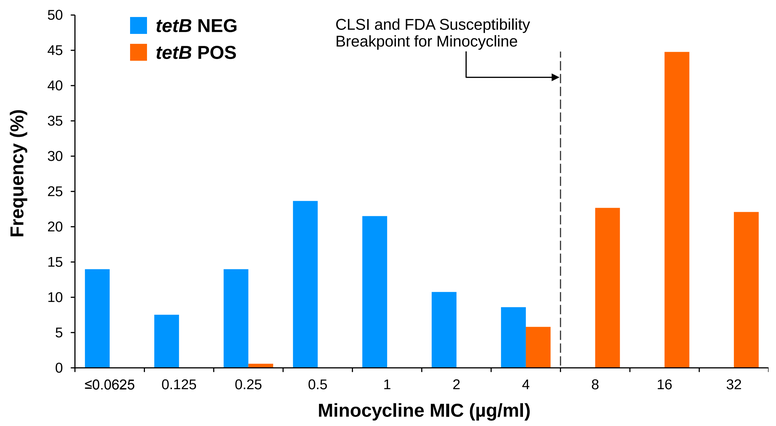

Table 2. Summary of minocycline susceptibility and *tetB* testing

	MINO MIC (µg/ml)		
	>4	≤4	Totals
tetB POS	154 (A)	11 (B)	165
tetB NEG	0 (C)	93 (D)	93
Total	154	104	258

- Of the 258 *A. baumannii* isolates, 165 and 93 isolates were found to be *tetB* positive and negative, respectively.
- Of the *tetB*-negative strains, all 93 (100%) were susceptible to minocycline (MIC ≤4 µg/ml, the FDA and CLSI susceptibility breakpoint)
- Of 165 *tetB*-positive strains, 154 were non-susceptible to minocycline (MIC >4 µg/ml)

Table 3. Summary of the statistical analysis

	Calculation	Result
Sensitivity for non-susc	A/(A+C) × 100	100%
Specificity	D/(D+B) × 100	89.4%
Positive Predictive value (PPV)	A/(A+B) × 100	93.3%
Negative Predictive Value (NPV)	D/(D+C) × 100	100%

Summary/Conclusions

- Table 1 shows MICs of ACB isolates tested. 44.6%, 68.8%, 93% and 80.6 % of isolates were resistant to minocycline, doxycycline, levofloxacin and meropenem, respectively.
- MIC frequency distributions of *tetB*-POS and *tetB*-NEG isolates showed a high degree of separation (Figure 1)
- *tetB* is highly associated with MICs above the current FDA and CLSI susceptible breakpoint (Tables 2 and 3).
- In view of the high sensitivity of *tetB* detection and minocycline non-susceptibility, *tetB* testing may be the basis for a surrogate susceptibility test (absence predicts susceptibility). Detection of the *tetB* gene would be particularly amenable to rapid testing. Further studies are warranted.

Disclaimers/Acknowledgements

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