Investigating aminoglycoside susceptibility in *Acinetobacter baumannii* and its relationship to oxidative stress

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**Introduction**

*Acinetobacter baumannii* is a Gram-negative opportunistic pathogen that is one of the major causes of hospital-acquired infections in the current healthcare system (Moubareck and Halat 2020).

- *A. baumannii* is associated with high mortality due to many strains achieving multidrug resistance (Moubareck and Halat 2020).
- Previous work demonstrated that deletion of catalase genes *katE* and *katG* in *A. baumannii* increased the cells’ resistance to aminoglycoside antibiotics; an increase in expression of the adeAB RND efflux pump was observed simultaneously (Kainth 2021).
- Gene knockout experiments deleting *katE*, *katG*, and *adeAB* genes in *A. baumannii* may help confirm a causal relationship between catalase gene deletion and adeAB efflux pump expression increase.
- Deletions were completed using either electroporation or conjugation methods to incorporate the non-replicative pMO31 plasmid backbone into the *A. baumannii* genome.
- The pMO31 plasmid is prepared by SOEing PCR and maintained in *E. coli* strains grown on LB plates containing gentamicin.
- Investigation of the effects of these deletions on aminoglycoside antibiotic susceptibility of *A. baumannii* strains was performed using minimum inhibitory concentration (MIC) tests.

**Objectives**

- To generate *A. baumannii* strains with various combinations of *katE*, *katG*, and *adeAB* gene deletions.
- To use MIC tests to compare aminoglycoside antibiotic susceptibilities between *A. baumannii* strains.

**Implications**

- Confirming a causal relationship between catalase gene deletion and adeAB efflux pump expression increase may help to elucidate some of the mechanisms of antibiotic resistance in *A. baumannii*.
- This information may be applicable to the goal of creating new treatments for this dangerous pathogen.

**Methods**

- **Gene deletion protocol** for *Acinetobacter baumannii* strains.

**Results**

- Obtained 3 potential deletion strains that grew consistently on gentamicin but not kanamycin plates during the second crosspatch but was not able to screen these strains yet due to ineffective PCR primers.
- Completed MIC tests for ATCC17978 (wild type), AB155 (*katG*), AB188 (*katE*), and AB189 (*adeAB*, *katE*) strains in the presence of kanamycin in one condition and in the presence of kanamycin + 5mM Ascorbic acid (thought to remove reactive oxygen species) in the second condition.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Strain</th>
<th>MIC with Kan</th>
<th>MIC with Kan + Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ATCC17978</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>AB188 (<em>katE</em>)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>ATCC17978</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>AB188 (<em>katE</em>)</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>ATCC17978</td>
<td>0.5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>AB155 (<em>katG</em>)</td>
<td>0.5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>AB189 (<em>adeAB;</em></td>
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<td>16</td>
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<tr>
<td></td>
<td><em>katG</em>)</td>
<td>0.5</td>
<td>46</td>
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<td>4</td>
<td>ATCC17978</td>
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<tr>
<td></td>
<td>AB155 (<em>adeAB;</em></td>
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<td><em>katG</em>)</td>
<td>1024</td>
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</tbody>
</table>

**Conclusion**

- Potential deletion strains with ATCC17978Δ*adeABkatG*, ATCC17978Δ*adeABkatE*, and ATCC17978Δ*adeABkatEv* genotypes generated.
- MIC results for ATCC17978 in the presence of kanamycin and ascorbic acid led to an unexpected increase in MIC that may be explained by hypotheses in the literature.
- RT-qPCR experiment designed to test some of these hypotheses and explore how ascorbic acid affects gene expression.

**Future Work**

- Complete the generation of strains with various combinations of the described genes deleted.
- Successfully screen these strains to confirm knockouts using PCR and gel electrophoresis.
- Carry out pLPF technique on strains containing cassettes to excise the gentamicin resistance marker.
- Test antibiotic susceptibility using MIC tests without resistance markers present.
- Finish qPCR experiment and interpret results to better understand differences in my MIC results compared to the expected results.

**References**


Goswami, M., Mangoli, T. 2021. Investigation into the regulatory mechanisms of resistance-nodulation-diviion efflux pumps in *Acinetobacter spp.* Masters thesis, University of Manitoba, Manitoba, MB.


**Acknowledgements**

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