Transcriptomic Changes in MRSA upon treatment with a Small Molecule Adjuvant

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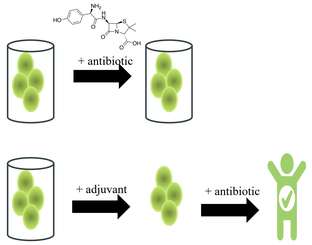
Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major public health concern, as it has developed resistance to our existing arsenal of antibiotics. This creates a limited amount of treatment options for antibiotic resistant bacteria. One way to combat this resistance is with an adjuvant along with an antibiotic. An adjuvant is a compound that has no antibiotic activity on its own, but when it is present with the antibiotic, repotentiation of the antibiotic occurs. Work in the Blackledge lab has developed numerous adjuvants including compound S8.



Transcription is the process of creating RNA from DNA. The transcriptome contains all the RNA transcripts present in an organism, and analyzing the transcriptome can illustrate gene changes such as up or down regulation. These changes can show what the treatment is targeting and to what extent.

To determine the molecular mechanism of compound S8, RNA from MRSA 43300 was isolated and sent off to Novogene for RNA sequencing and analysis.

It was hypothesized that treatment with both oxacillin and compound S8 would result in either up regulation or down regulation of genes associated with antibiotic resistance.

Methods

- Purified total RNA from MRSA 43300 and analyzed concentration and purity using Nanodrop
- Sent off RNA samples to Novogene for RNA sequencing and analysis

Results

Figure 1: Volcano plot of genes comparing treatment with oxacillin and co-treatment illustrating significance of 176 up regulated genes and 233 down regulated genes

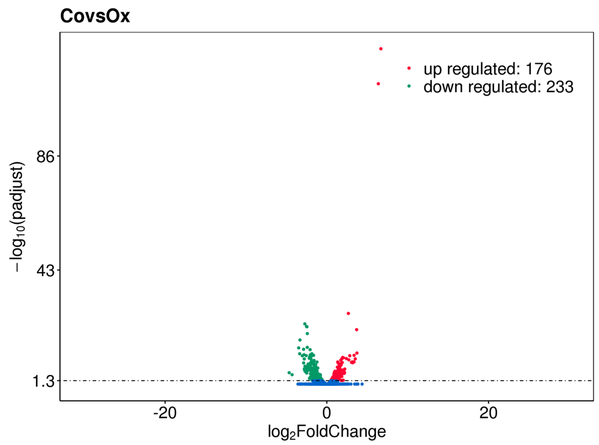


Table 1: Comparison of Log2 fold changes in key resistance genes between treatment with oxacillin and co-treatment from RNA sequencing results and previous RT-qPCR results

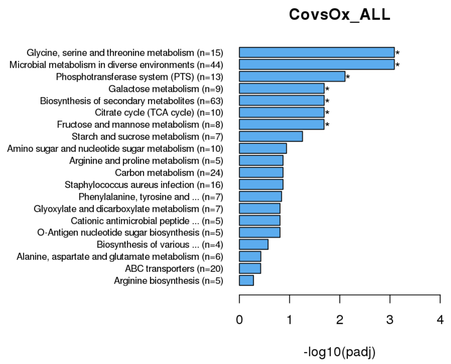
Gene	RNA seq Log2 fold Change	RT-qPCR Log2 Fold Change
mecA	-2.70	-2.00
mecI	-0.88	-0.36

Negative Log2 fold changes indicate a decrease in expression and positive Log2 fold changes indicate an increase expression.

Table 2: Most significant Log2 Fold Changes in Genes with the corresponding p-value when comparing treatment with oxacillin and co-treatment

Gene	Log2 Fold Change	P-value
hrtB	6.684	3.67E-127
hrtA	6.369	5.60E-114
sasA	2.66	2.31E-27
lrgB	-4.66	5.11E-5
lrgA	-4.30	2.93E-4

Figure 2: KEGG Pathway Analysis illustrates number of genes that compound S8 is targeting (n) within specific metabolic pathways as well as the statistical significance



Conclusion

- 176 genes were upregulated upon co-treatment, 2 of which that were extremely statistically significant, while 233 genes were down regulated upon co-treatment
- *hrtB* and *hrtA* genes had the most statistically significant changes in Log2 fold change when oxacillin treatment was compared to untreated. These genes are associated with hemin transport.
- *lrgB* and *lrgA* had the most negative log2 fold changes when oxacillin treatment was compared to untreated. These genes are associated with cell lysis.
- *mecA* and *mecI* genes had similar Log2 fold changes between treatment with oxacillin and co-treatment in both the RNA sequencing analysis and previous RT-qPCR experiments, validating these gene expression changes
- Co-treatment with compound S8 and oxacillin targets multiple genes that are related to metabolism and the citrate cycle, indicating mechanism of action

Future Directions

- Continue to compare RT-qPCR results with RNA sequencing findings
- Complete RT-qPCR analysis studying genes that show significant changes upon co-treatment
- Sequence strain USA300

Acknowledgments

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References

Gillard, K; Miller, H.B.; Blackledge M.S. (2018) Tricyclic Amine Antidepressants Suppress b-lactam Resistance in Methicillin-Resistant Staphylococcus aureus (MRSA) by Repressing mRNA Levels of Key Resistance Genes. *Chemical Biology and Drug Design.* 92(5):1822-1829. doi: 10.1111/cbdd.133