Alive or just detectable? Rethinking *Mycoplasma genitalium* testing with new viability markers

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Background:
Antimicrobial resistant (AMR) *Mycoplasma genitalium* poses an increasing public health threat both in Australia and globally. As resistance rises, test-of-cure (TOC) is increasingly recommended to confirm treatment success. However, current molecular TOCs cannot distinguish viable *M. genitalium* from non-viable and non-replicating bacteria present after successful treatment, leading to false positive results and potential overtreatment. We aimed to address this issue by applying the power of transcriptomics to differentiate actively replicating *M. genitalium* from stressed/non-replicating *M. genitalium*, with the goal of identifying robust markers of bacterial viability.

Methods:
Four *M. genitalium* strains were assessed, each with varying AMR profiles and geographic origins. To identify markers of viability, we compared actively replicating *M. gentialium* with non-actively replicating populations generated under two conditions: antibiotic exposure (moxifloxacin) and nutrient starvation. RNA was extracted from each condition and analysed using high-depth whole transcriptomic sequencing. Sequencing data was processed using publicly available bioinformatics tools and pipelines, with transcriptomic reads mapped to the *M. genitalium* reference genome and gene expression was then quantified. Differential gene expression was then assessed by comparing expression levels among replicating and non-replicating *M. genitalium* populations.

Results:
We have identified 54 transcripts that differ in expression between actively replicating organisms and those exposed to antibiotic treatment or nutrient deprivation. Notably, ten transcripts were consistently up- or down-regulated among both antibiotic treated and nutrient starved conditions, when compared to untreated controls.

Conclusion:
These ten markers may be associated with bacterial replication and further validation will assess their utility as markers of viability. If validated, these markers could be

translated into a practical assay to distinguish viable from non-viable organisms, improving the accuracy of test-of-cure and reducing overuse of antibiotics.

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None

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