Dead or alive: new precision tools for determining *Mycoplasma genitalium* and *Neisseria gonorrhoeae* viability

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Background:
Neisseria gonorrhoeae and *Mycoplasma genitalium* are sexually transmitted infections, which combined infect more than 150 million people each year worldwide. Both microbes are rapidly acquiring antimicrobial resistance (AMR) to all available treatments. To combat the rise in AMR, high-throughput methods that accurately assess *N. gonorrhoeae* and *M. genitalium* viability and replication patterns after treatment with antibiotics are sorely needed. Current methods of identifying and quantifying these microbes, such as PCR and culture either lack specificity for live and replicating cells or are not high throughput.

Methods:
To assess the viability and replication of *N. gonorrhoeae* and *M. genitalium*, we created a flow cytometry method. We utilised a membrane exclusion dye and a cell trace dye to differentiate live and dead cells and replicating and non-replicating cells, respectively. We used this method to analyse both untreated bacteria and bacteria treated via nutrient starvation or antibiotics, to identify replicating and non-replicating bacterial populations. The quantitative outcomes from flow cytometry were compared to qPCR assays and culture-based approaches.

Results:
Flow cytometric analysis showed that, when nutrient deprived or exposed to antibiotics, the proportion of non-replicating *M. genitalium* and *N. gonorrhoeae* cells increased by 54.4% and 29.4%, respectively. Additionally, where qPCR could not establish a significant difference in cycle threshold ($P>0.05$) between replicating and non-replicating (treated) bacterial populations, flow cytometry consistently found a significant difference ($P<0.01$) in live cells/mL, demonstrating its superior specificity.

Conclusion:
This flow cytometry method makes it possible to differentiate live and actively replicating *M. genitalium* and *N. gonorrhoeae* from those that are non-replicating or killed, including those treated with standard of care antibiotics. Utilising flow cytometry will enable future investigations of these antimicrobial resistant STIs at the single cell and population level, to better understand treatment failure and as a benchmark to assess the efficacy of novel antimicrobial compounds.

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