A 2D in-vitro cell-based model for antimicrobial susceptibility testing against oral gonorrhoea

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Background:
Treatments for *Neisseria gonorrhoeae* (NG) remain scarce as NG becomes increasingly resistant with higher treatment failure in the oropharynx. However, little is known about oral NG infection dynamics. We developed the world's first in-vitro co-culture model for NG with human oral cells to understand infection dynamics and to rapidly screen for new antimicrobials to treat oral NG.

Methods:
In our preliminary model, we infected three oral cell subtypes – gingiva, tonsils and floor-of-the-mouth with either an antimicrobial-susceptible (FA1090) or an antimicrobial-resistant (FC428) strain of NG. We assessed tissue invasion (colony forming units; CFUs) and intracellular kill following exposure to tetracycline, ciprofloxacin, azithromycin, ceftriaxone, cefixime and gentamicin at 1, 2, 3x MIC after 30, 60 and 120 minutes post-infection and at 15 and 30 minutes prior to infection to validate the model. Pre- and post-treating cells with tetracycline was done to simulate doxycycline pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP). Post-treatment cells were washed with gentamicin, cells lysed for CFU counting, and internalized bacteria were visualized using confocal microscopy.

Results:
NG infected all cell types, with highest CFUs observed in the tonsils and gingiva for both strains. Gentamicin showed ineffective intracellular clearing of NG, while other antibiotics demonstrated clearing of NG, with significant CFU reductions within 2 hours. These findings reflect clinical observations, and strongly support the validation of our model. Data from tetracycline treatment suggests that pre-treatment of cells (DoxyPreP) was more effective against FC428 strain (MIC90 2mcg/mL) vs post-treatment of cells (DoxyPEP).

Conclusion:
We have developed a validated 2D-model of oral NG infection, testing currently recommended treatments in three different oral cell types, demonstrating our results align with clinical findings. With this model, we will study oral NG infection dynamics, screen novel antimicrobials to find new treatments for oral NG and to support the development of a 3D model.

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