350 words, 1 figure

Title:
Bacterial Impedance Cytometry (BIC) - Gaining insights into bacterial responses to antimicrobials

Background:
Current methods to monitor bacterial responses to antimicrobials are time consuming, costly, require cells to be fixed or manipulated and track cells at whole population level. BIC is a novel, fast, simple, label-free, single cell, phenotypic approach to monitor the tiny electrical changes taking place in bacteria in response to antimicrobial agents. Using this technology to understand the responses of WHO priority pathogens, including *Enterobacterales*, *Staphylococcus* and *Neisseria* against antibiotics, phage and novel antimicrobial compounds, has given us insights into modes of action, dose-response and resistance.

Method:
$5x10^5$cfu/mL logarithmically growing cells were exposed to the antimicrobials at various concentrations for 2 hours at 37˚C static. Timings and media were adjusted for different organisms. Cells were diluted in saline and impedance of individual cells at 5 and 40mHz recorded (ca. 5000 cells/sample). Changes in impedance compared to an unexposed control were analysed using various metrics. Results were compared to those from current gold standard methods.

Results:
The cell count of 58 strains of *Enterobacterales* exposed to breakpoint concentrations of 8 antibiotics showed 100% concordance for susceptible/resistant with gold standard MICs when MIC was ± 1 log2 (doubling dilution) different to the breakpoint concentration. Data from a clinical concordance study will be provided to show performance in a clinical setting.

Electronic MICs (eMIC), where bacteria are exposed to doubling dilutions of antibiotics, showed various impedance signatures at sub-MIC concentrations. These varied depending on the mode of action of the antibiotic, allowing insight into the stress responses of the cells, linked to bacterial survival under antibiotic selection.

Neisseria gonorrhoeae similarly showed variation in impedance signature depending on mode of action of the antibiotic and susceptibility profile of the strain, allowing an Antimicrobial Susceptibility Test (AST) in <6 hours.

The phage infection cycle can be tracked using BIC, as the basis of rapid phage susceptibility testing within 2 hours and assessment of resistance emergence in <5 hours.

Conclusions:
BIC can be used as a fast, label-free, single-cell, low volume AST for diverse bacteria and antimicrobials, including additional information about bacterial responses to antimicrobials.

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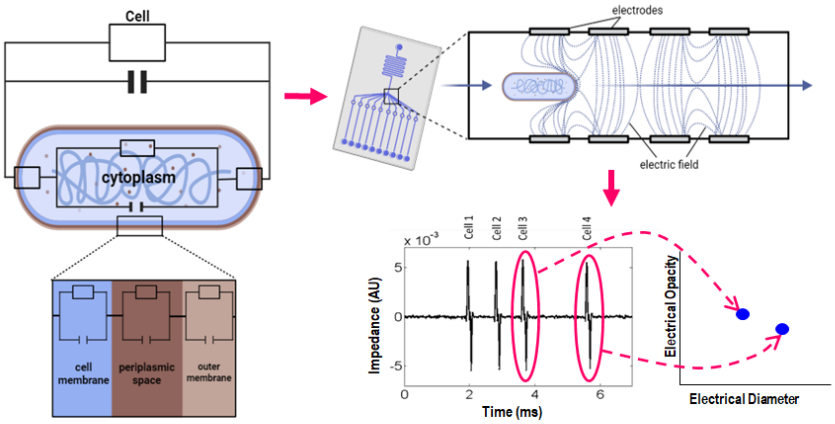

Figure 1: Bacterial Impedance Cytometry (BIC) measures the tiny electrical changes in individual cells upon exposure to antimicrobials.

A Gram-negative bacterium will impede an electrical current due to its resistance in membranes, periplasmic space, cytoplasm and size.

In BIC individual cells flow through a nanofluidic channel, where a 5 MHz and 40 MHz current is applied. Each event will have an electric read-out which can be shown as electrical opacity (measure of membrane/cell wall properties normalised to cell volume, measured at 40 MHz where the electrical properties of the cell wall and membrane are most apparent) vs electrical diameter (cube root of the impedance, proportional to diameter, measured at a frequency of 5 MHz).