Title: A fast, accurate antimicrobial susceptibility test (AST) for bacteria from urine samples

Background:
UTIs are a common form of infection and reason for antibiotic prescribing. Empirical prescribing is widespread due to the high incidence, burden of symptoms and the 24-48 hours needed for an AST. However, untreated UTIs can lead to complications and in severe cases to bacteraemia and death. In addition, resistance to frontline antibiotics is increasing, leading to increased treatment failure. There is, therefore, a need for a fast, accurate and cost-effective AST for urine samples.

The impedance-based Fast AST (iFAST)[1] can rapidly measure an antibiotic susceptibility profile by analysing the electrical properties of many single bacteria at high speed. The electrical properties of sensitive bacteria treated with frontline UTI antibiotics change significantly compared to untreated cells. Sensitivity to breakpoint concentrations of antibiotics is visible on the iFAST within two hours of exposure.

Method:
57 *Escherichia coli* and *Klebsiella pneumoniae* isolates were grown for 2 hours on Cystine Lactose Electrolyte Deficient agar plates at 37˚C. A loopful of bacteria was adjusted to 5 x 10^5cfu/mL in saline and exposed to EUCAST breakpoint concentrations of frontline UTI antibiotics: co-amoxiclav (2/8mg/L), amoxicillin (8mg/L), ceftazidime (1mg/L), cefalexin (16mg/L), ciprofloxacin (0.25mg/L), gentamicin (2mg/L), nitrofurantoin (64mg/L), trimethoprim (4mg/L) or a no antibiotic control in MH2 media at 37˚C. After two hours, 30µL of each culture was measured in the iFAST for 30 seconds and compared to the control. The results were compared to a gold standard microbroth dilution MIC on the same sample.

Results:
For >434 tests conducted, overall concordance with the gold standard method was 95.2%. The highest concordance was seen for ceftazidime (100%) and amoxicillin (100%), the lowest for cefalexin and nitrofurantoin (both 91%). All non-concordances showed an MIC of ± 1 log2 (doubling dilution) different to the breakpoint concentration. As this is within the normal technical variability of the EUCAST gold standard method, this can be interpreted as 100% concordance.

Conclusions:
iFAST has up to 100% concordance with current gold standard MIC methods and delivers the results straight from samples in <5 hours. This novel, fast, accurate and cost-effective AST could enable evidence-based antibiotic prescribing.

Reference:
[1] Spencer DC, Paton TF, Mulroney KT, Inglis TJJ, Sutton JM, Morgan H. A fast impedance-based antimicrobial susceptibility test. Nat Commun. 2020 Oct 21;11(1):5328. doi: 10.1038/s41467-020-18902-x. PMID: 33087704; PMCID: PMC7578651.

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Authors:
L. J. Bock[a], D. C. Spencer[b], A. Sinha[b], B. T. Martin[a,b], H. Morgan[b], J. M. Sutton[a]
[a] Technology Development Group, UK Health Protection Agency, Salisbury, SP4 0JG, United Kingdom
[b] Department of Electronics and Computer Science, University of Southampton, Hampshire SO17 1BJ, United Kingdom

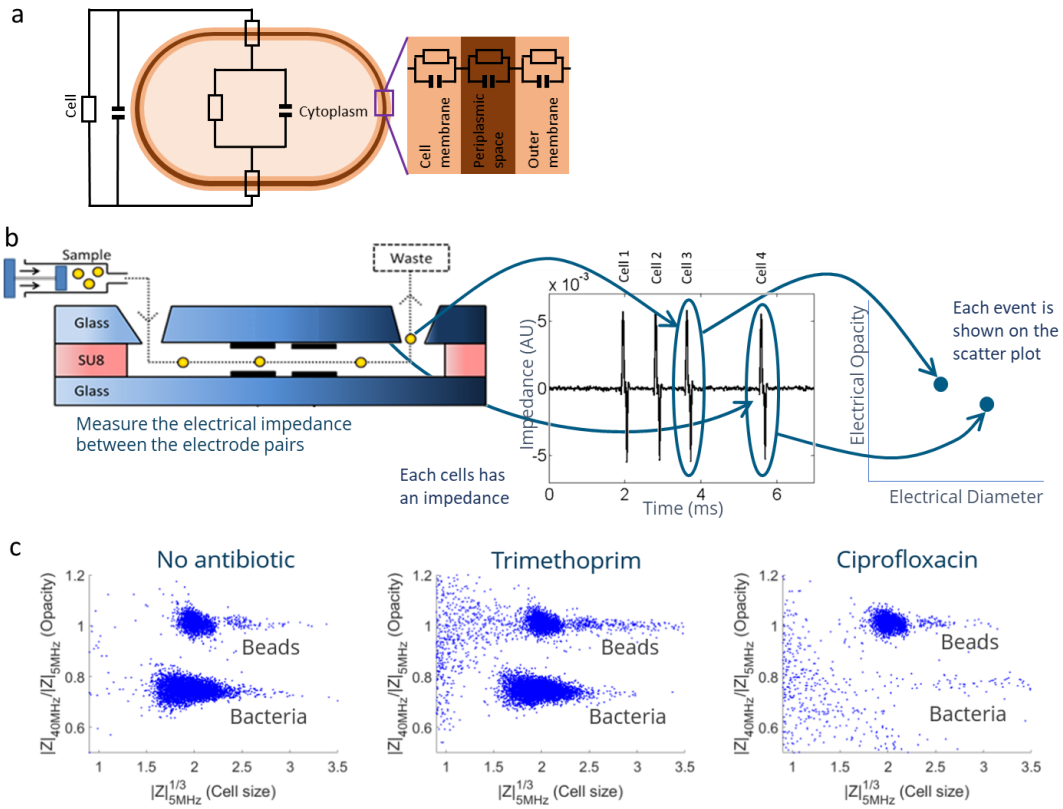

Figure 2: iFAST uses electrical impedance to measure antibiotic susceptibility.
a) A Gram-negative bacterium will impede an electrical current due to its resistance in membranes, periplasmic space, cytoplasm and size.
b) In the iFAST individual cells flow through a nanofluidic channel, where a 5 MHz 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).
c) Impedance scatter plot of *K. pneumoniae* exposed to trimethoprim (resistant) and ciprofloxacin (sensitive) breakpoint concentrations, together with 1.5µm diameter polystyrene beads that are used as reference particles. Following two hours of exposure sensitive cells are killed and no longer visible on the iFAST whereas resistant and control populations stay visible.