Rapid Estimation of Antibiotic Efficacy for Low Bacterial Inoculums using Multi-frequency Impedance Measurements

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Abstract

Background: In order to obtain Minimum Inhibitory Concentrations (MICs), antibiotics are, by convention, tested against turbidometrically standard suspensions containing ~$5x10^5$ CFU/ml of bacteria. This not only fails to accurately represent the clinical situation, where loads range from < 10CFU/ml for septicemia to >107CFU/ml for meningitis, but introduces a delay of ~1day to allow for the preparation of the turbidometric standard from colonies

Methods**:** We use a multifrequency impedance measurement method to observe in real time the effect of antibiotics on bacteria present at loads of ~ 10^3 CFU/ml. Applied high frequency (100KHz to 100MHz) AC voltages leads to charge-buildup at the membranes of living cells (due to their membrane potential), contributing to a "bulk capacitance" (Cb) in the suspension. An increase in cell number (bacterial proliferation) results in an increase in Cb, whereas cell-death leads to a loss of membrane potential (decrease in Cb). Cb remains unchanged in the case of bacterial stasis. This allows us to directly observe the mode of action of the antibiotic (whether bactericidal or bacteriostatic) and determine MIC.

Results**:** At bacterial loads of ~10^3 CFU/ml, the MICs of gentamicin and amikacin against *Pseudomonas aeruginosa* ATCC-27853 were 0.5 and 2 mg/l, whereas the MICs of ampicillin and chloramphenicol against *Escherichia coli* ATCC-25922 were 8 and 2 mg/l, respectively. With the exception of gentamicin where the recorded MIC was lower, all MICs were within the expected range for the combination of strain and antibiotic (as measured for loads of ~10^5 CFU/ml). Also, amikacin was observed to act in a bactericidal manner at its MICs for loads 10^3 CFU/ml, whereas its action is bacteriostatic at loads of ~10^5 CFU/ml.

Conclusions**:** Our method, which enables direct observation of antibiotic-bacteria interaction at low bacterial loads (~10^3 CFU/ml), can be used to determine the presence of an "inoculum effect" for low bacterial loads. This, coupled with the time saved by avoiding the day-long process involved in preparing turbidometric standards, can have clinical benefits.