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 >10^7CFU/ml for meningitis, but introduces a delay of ~1 day 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” (C_b) in the suspension. An increase in cell number (bacterial proliferation) results in an increase in C_b, whereas cell-death leads to a loss of membrane potential (decrease in C_b). C_b 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.