

Novel Electrical Method for the Rapid Determination of Minimum Inhibitory Concentration (MIC) and Assay of Bactericidal/Bacteriostatic Activity

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Abstract

We present a rapid (4-hr) electrical method for Antibiotic Susceptibility Testing that not only yields the MIC of candidate antibiotics, but also simultaneously determines the antibiotics' effect on the bacteria (bactericidal/bacteriostatic). Unlike conventional "impedance microbiology" methods that rely on measuring the effects of bacterial metabolism on the conductance/impedance of the suspension at a single chosen frequency, our method uses measurements at 500 frequencies between 1 KHz and 100 MHz to estimate the amount of electric charge stored due to charge-polarization at intact cell-membranes of living bacteria (the suspension "bulk capacitance"). By doing so, we are able to track the number of live bacteria in suspensions as the observations are taken (every 1 hour). It thus determines whether the numbers of viable bacteria present is increasing (bacteria proliferating in presence of antibiotic), decreasing (bacteria being killed) or holding steady (bacterial numbers held static). Three well-characterized bacterial strains (*E. coli* ATCC-25922, *S. aureus* ATCC-29213 and *P. aeruginosa* ATCC-27853) were tested against a range of concentrations (0 to 128 mg/l) of known static and cidal antibiotics. For each sample (bacterial strain at a given concentration of antibiotic), statistical analysis of the "bulk capacitance" values, recorded over 4 hours was used to determine whether the bacteria were proliferating, being killed, or being held static. The minimum concentration of antibiotic for which the bacteria were killed or failed to proliferate is considered the Minimum Inhibitory Concentration (MIC). MICs obtained fell within the expected range for the strains tested, and "static" and "cidal" antibiotics were correctly identified. This method thus demonstrates the potential to provide in 4 hrs, clinically relevant information such as the MIC of bacterial strains (that currently take up to 2 days) and the mode of action (static/cidal) that currently takes an additional day.

Keywords: Antibiotic susceptibility; Detection; MIC; Bactericidal; Bacteriostatic; Microfluidics

Introduction

In a number of clinical situations, knowing the antibiotic susceptibility profile of the particular pathogen causing the infection (in particular the MIC of various candidate antibiotics) can help determine the optimum treatment protocol. For instance, it has been reported that for certain antibiotics (β -lactams, macrolides, clindamycin and linezolid), the clinical efficacy is strongly correlated with the duration for which their concentrations in the serum was above their respective MICs, whereas for others (aminoglycosides and fluoroquinolones), the ratio of the peak serum concentration to the MIC is the major determinant of efficacy [1]. In certain, more critical situations (such as endocarditis and meningitis), it may be desired to administer antibiotics at bactericidal doses that kill the infecting organism, as opposed to bacteriostatic doses that merely prevent the organism from proliferating further [2]. In other cases, such as streptococcal and clostridial gangrene, it is more preferable to use bacteriostatic drugs, since cidal drugs can cause the dying cells to release internal toxins, which may further aggravate the morbidity [2]. Since drugs that are bactericidal for one organism may be bacteriostatic for other organisms, or other strains of the same organism [2], it is not always possible to predict the mode of action for a given antibiotic.

Further, it can take relatively long time (upto 2 days) to obtain MICs of candidate antibiotics to particular strain [3], and an additional day to obtain information regarding the bactericidal/bacteriostatic nature of a given antibiotic's activity at various concentrations above its MIC [4]. Cutting down the time needed to determine MICs and to assay for bacteriostatic/bactericidal activity could help the clinician formulate more effective treatment protocols and achieve better patient

outcomes. In addition, a method that simultaneously indicates the mode of action of the antibiotic (cidal or static) could be of added value to the clinician.

MICs are currently determined using disc-diffusion or broth-dilution (macro or micro) methods. While disc-diffusion remains a largely manual technique, broth-dilution (esp. micro-dilution) has been automated in the recent past to reduce labor costs and preparation time, and has emerged as the technique of choice for large clinical microbiology labs. These automated Antibiotic Susceptibility Testing (AST) instruments rely on a variety of methods to determine the occurrence of bacterial proliferation (or lack thereof) in the presence of various concentrations of the candidate antibiotics. For instance, VITEKTM (from Biomeriux) uses an increase in solution turbidity as a measure of an increase in bacterial concentration [5], whereas the PhoenixTM (Becton-Dickinson) and the Microscan WalkAwayTM (Dade Microscan) systems use fluorimetric/colorimetric methods to detect ongoing metabolism (redox reactions) of surviving bacteria [5,6]. These systems suffer from two major drawbacks. Firstly, the time

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needed to obtain susceptibility profiles is still rather long, typically 12-24 hours [5,7]. Secondly, they do not tell the user whether the action of the antibiotic is bacteriostatic or bactericidal. To determine the mode of action, the suspensions of bacteria exposed to different concentrations of antibiotic for 12-24 hours are plated on nutrient-agar plates and incubated for an additional 24 hours. Bacteria that survived the exposure to antibiotics yield colonies, and the numbers of colonies observed for the different concentrations are used to determine the mode of action of the antibiotic [8].

Various other approaches are being investigated to reduce the time needed to obtain MIC values. Newer methods being developed include those using dielectrophoresis (DEP) [9], microfluidic incubation [3], magnetic bead rotation sensors [10]. However each of these methods has its own limitations. The DEP-based method assays for the effect of the antibiotic by monitoring elongation of the bacterial cells (that causes a change in their DEP properties). This method was demonstrated for β -lactam antibiotics on gram-negative bacteria (*E. coli* and *Klebsiella pneumoniae*). It is thus very specific, and possibly cannot be generalized to all antibiotic-bacteria combinations. The method based on sensing magnetic-bead rotation needs an antibody specific to the bacterium being investigated to be conjugated to the magnetic beads, using which the bacteria of interest adhere to the magnetic beads (sensing platforms), again making an individual test specific to a particular bacterium. It may be noted that both VITEK™ and Pheonix™ systems do not require prior knowledge of bacterial ID, allowing AST to be performed in parallel with bacterial identification. Time saved by using these methods would be of less clinical value if they can be initiated only after the infecting bacteria have been identified. The microfluidic incubation method uses optical density (OD) to monitor bacterial growth (or lack thereof) in 10 μ l micro-reactors. It is thus a microfluidic analog of the classic microdilution method, with the added advantage of a shorter assay time. However, like the commercially available microdilution instruments/methods, it, along with the other emerging techniques mentioned above, is not able to distinguish between the bactericidal and bacteriostatic action of the antibiotics. In the present work, we demonstrate a method that is not only generic (like those using turbidity and fluorescence), but also

significantly faster and capable of providing information regarding the mode of action of the antibiotic.

Theory

At the core of our method lies an electrical technique that tracks the number of live bacteria in suspensions [11-12]. Our method utilizes the ability of viable bacterial cells to become “polarized” in the presence of AC electric field. This polarization leads to buildup of charges across the intact membrane of viable bacterial cells [13] and hence these cells effectively behave like electrical capacitors. As the viable bacteria reproduce in a suspension, greater numbers of bacteria result in an increase in the charge stored in the interior of a suspension (the “Bulk” or “Medium” Capacitance (C_b)). This principle has been used to determine the presence of viable bacteria in food samples [11-12] and blood cultures [12] 4-10 times faster than existing methods that typically rely on detecting effects of bacterial metabolism such as changes in O_2/CO_2 levels, pH, solution conductivity etc.

Among the various methods used to detect bacterial proliferation indirectly (through the effects of bacterial metabolism on solution properties) lies a class of electrical methods called “impedance microbiology” [14]. Viable bacteria break down sugars to more conductive species such as lactate and carbonate. This makes the solution more conductive, decreasing the bulk resistance (R_b of figure 1b) [15] and total Impedance (Z of Figure 1b) [16]. Interfacial capacitance (C_e of figure 1b) is also affected [17] since the ions in the double-layer are in electro-chemical equilibrium with the bulk. While “impedance microbiology” is used in certain commercial devices (such as the RABIT [14]), to detect viable bacteria in foods, it has not been used commercially for Antibiotic Susceptibility Testing. Even if it were used, it would, like other methods that detect bacterial proliferation indirectly via the effects of bacterial metabolism, not be able to distinguish between bacteriostatic and bactericidal action of antibiotics.

Until our work [18], others had failed to detect changes in C_b of proliferating bacterial samples, although they had tried to measure it [19]. In fact, a recent (2008) review [20] summarized the conventional wisdom thus:

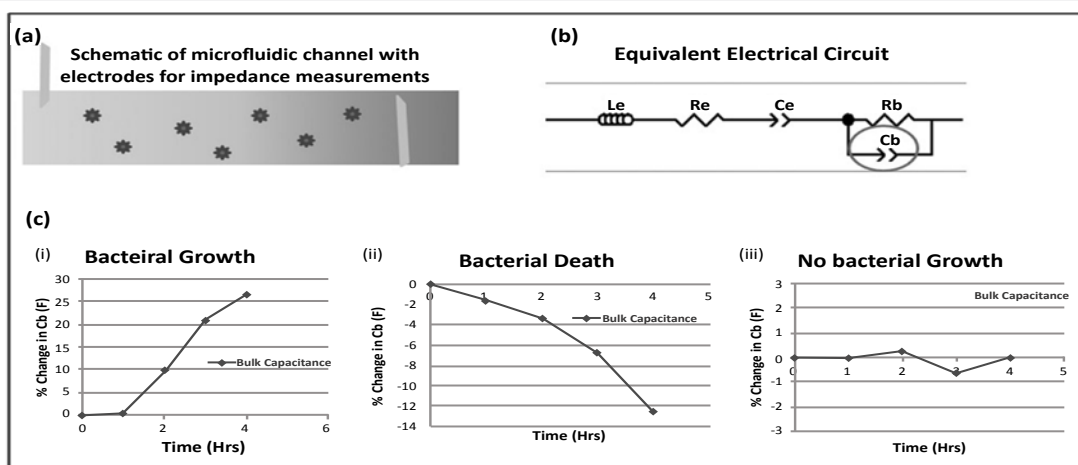


Figure 1: (a) Gives the schematic representation of the suspension of bacteria in media inside the micro-channel in contact with the metal electrodes (b) Equivalent circuit used to model the electrical behavior of the same. The circuit represents the Inductance (L_e), Resistance (R_e) and the Capacitance (C_e) at the electrodes along with the Resistance (R_b) and Capacitance (C_b) of the bulk of the suspension containing bacteria (c) Gives the typical plots of the C_b values (obtained after fitting the data to the equivalent circuit in (b)) as a function of time where (i) indicates no change in bulk capacitance values as the bacteria does not grow over time (ii) indicates the increase in the value of bulk capacitance as bacterial concentration increases (iii) indicates a decrease in the bulk capacitance values as bacterial concentration decreases over time.

“Geometric capacitance is due to the solution between the electrodes and depends on medium permittivity and the distance between the electrodes. Because of its small value, in the picofarad range, it can usually be neglected in the measurement frequencies used in biosensor applications”

Our prior innovation has been to measure this usually neglected parameter, and show that by tracking it over time; we can detect viable bacteria in the sample much faster. In the current work, we seek to demonstrate that it can also be used to rapidly determine whether bacteria, in a given suspension (in the presence of a certain concentration of antibiotic), are proliferating, dying, or being held static.

This is because cell death is accompanied by loss of membrane potential and electrical polarization [21]. As a consequence, the death of some cells in the suspension being monitored leads to a measurable decrease in the value of its C_b . For this study, our hypothesis was the following: For suspensions containing cells of a particular bacterial strain and antibiotic at known concentration, if the bacteria are able to grow, then the suspension C_b will increase with time, whereas if the bacterial cells die, the value of C_b will decrease over time. Further, if the bacterial cells are not actively growing or dying, then the value of C_b will remain essentially unchanged. This would occur because the measured C_b of the suspension at any given point in time is the sum of the bulk capacitance of the solution in which the bacteria are suspended (C_{b_soln}) and that of the viable bacteria in suspension. The number of viable bacteria in the system (n) is given by

$$n = n_0 e^{kt} \quad (1)$$

where n_0 is the initial number of bacteria present and k is the specific growth/death rate (positive for systems in which cell number is increasing and negative for those in which cell number is decreasing). Thus, the value of the bulk capacitance of the suspension, as a function of time, would be given by

$$C_b = C_{b_soln} + (n_0 C_{b_bac} \times e^{kt}) \quad (2)$$

where

C_b = measured bulk capacitance of suspension

C_{b_soln} = capacitance of the solution alone

C_{b_bac} = capacitance of individual bacteria

n_0 = number of bacteria in the solution at time $t=0$

k = specific growth/death rate

t = time over which measurements are made

Thus, in theory, if a set of C_b values (as a function of time) is fit to Equation (2) above, and various parameters estimated, then the value of k will be positive for systems with bacterial proliferation, negative for systems in which bacteria are dying off, and be (statistically) equal to zero for systems in which bacteria are prevented from reproducing, but are not being killed. Hence this method, when applied to samples with different concentrations of antibiotics, can be used to not only determine Minimum Inhibitory Concentrations (MICs), but also to distinguish between bacteriostatic and bactericidal action of antibiotics.

Materials and Methods

Bacterial cultures: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853) (bacterial strains whose susceptibility to various antibiotics have been

extensively studied [22-27]) are cultured for 12-48 hrs in Tryptic Soy Broth (TSB) to obtain log cultures. These strains were specifically selected as they are three of the commonly used control ATCC strains for AST studies [27].

Sample preparation: The cultures are centrifuged, and the pellets (bacterial cells) are re-suspended in TSB. OD of these samples is measured against control (TSB without bacteria) using a UV-Vis spectrophotometer (UV 1650PC, Shimadzu Scientific Inc) at 625 nm and the samples are adjusted by diluting with TSB to have an OD in 0.08-0.13 range. These samples which, after the OD adjustments, are expected to have a bacterial concentration of $\sim 10^8$ CFU/ml [27], are then serially diluted in sterile Mueller Hinton (MH) broth to obtain a bacterial concentration of 106 CFU/ml. To the latter, equal volumes of antibiotic solutions in MH broth are added to obtain bacterial concentrations of $\sim 5 \times 10^5$ CFU/ml (the recommended inoculation concentration for the broth macrodilution AST). The antibiotic dilutions used in the experiments are prepared in sterile MH broth at twice the concentrations of the desired final antibiotic concentrations, as the solution will be inoculated with equal amount of 10^6 CFU/ml bacterial solutions. 5 ml each of the 10^6 CFU/ml bacterial samples are added to 5ml of prepared antibiotic solutions to give a final bacterial concentration of $\sim 5 \times 10^5$ CFU/ml in each tube with antibiotic concentrations of 0.5 mg/l, 1 mg/l, 2 mg/l, 4 mg/l, 8 mg/l, 16 mg/l, 32 mg/l, 64 mg/l and 128 mg/l. The concentration of antibiotics chosen are the standard concentrations that the Clinical and Laboratory Standards Institute (CLSI) recommends for testing unknown strains against candidate antibiotics [28]. The combination of bacteria-antibiotic pairs used in the experiments is given in table 2. With each bacterium-antibiotic pair, a control sample is also incubated with 5×10^5 CFU/ml bacterial concentrations but without the antibiotic. These tubes are incubated on a shaking platform (Fisher Scientific Nutating Mixer) within an incubator ((Fisher 637D Isotemp Incubator) at 37°C for 4 hours.

Multi-frequency Impedance Measurements: Every hour, we draw a small (250 μ l) aliquot from the culture, and estimate the bulk capacitance of the sample using previously described protocols [11-12]. Briefly, the aliquot is injected into the microfluidic cassette (Figure 1c). The gold electrodes on the cassette are connected via a 16047E connector an Agilent 4294A Precision Impedance Analyzer (Agilent Technologies) and electrical impedance measurements are taken using a 500 mVAC source over a frequency range of 1KHz to 100 MHz (200 logarithmically equi-spaced frequency points). Parallel 100 μ l samples are also plated out (after appropriate serial dilution) at the same point in time to verify bacterial concentration (Not shown in results). Electrical Impedance data obtained from the analyzer is in the form of Resistance (R) and Reactance (X) values at 200 frequencies (ω) between 1 KHz and 100 MHz.

Data Analysis: Our circuit model for the electrical behavior of the system consisting bacterial suspension in a micro-channel [as shown schematically in figure 1(a)] is shown in figure 1(b). As seen in the figure, it takes into account lead wire inductance (L_w), electrode resistance (R_e), charge storage at the electrode-solution interface (C_e), bulk-solution resistance (R_b) and capacitance (charge storage) in the bulk (C_b) [18,29]. To account for the non-ideal (non-instantaneous) behavior of the charge storage elements, at both the electrode and the bulk, we have, like others before us [30], used Constant Phase Elements (CPEs) instead of ideal capacitors. The magnitude of the CPE may be considered to represent the degree of charge storage within the element.

The values of Resistance and Reactance at different frequencies are fit to the circuit shown using commercially available software (Z-view™), and an estimate for the magnitude of the CPE (measure of the bulk capacitance) is obtained. Examples of fit to the data obtained are shown in figure 2.

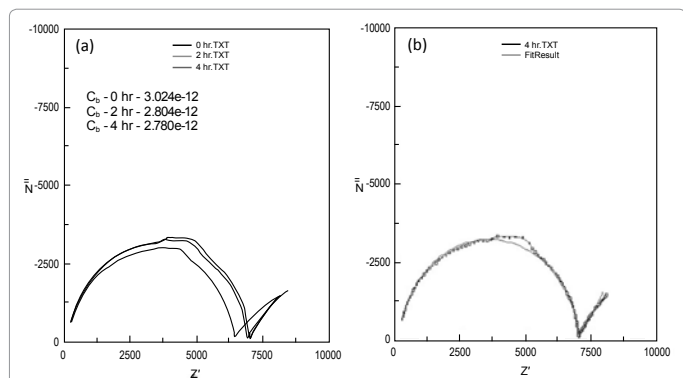


Figure 2: Represents the sample fits of Impedance (Z) vs. Frequency (ω) data to the proposed circuit model using the software ZView™. (a) Represents the plot of reactance (Z'') vs. resistance (Z'), for 0 hr, 2 hr and 4 hr for *E. coli* in Ampicillin at 8 mg/ml as a sample data set (b) Represents the sample fit (smooth line) using the circuit model for the 4 hr reading (line with dots) of *E. coli* in Ampicillin at 8 mg/ml.

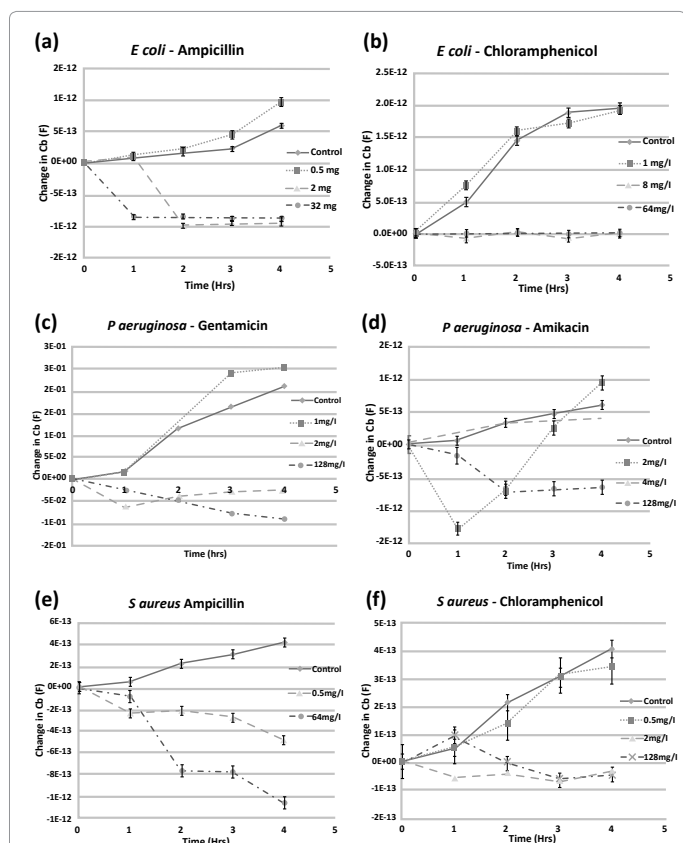


Figure 3: Plots showing how the value of the bulk capacitance of bacterial suspensions change over time for *E. coli* exposed to (a) Ampicillin and (b) Chloramphenicol, (c) *P. aeruginosa* exposed to Gentamicin and (d) Amikacin and (e) *S. aureus* exposed to Ampicillin and (f) Chloramphenicol at different concentrations. The antibiotics used on the left column (a), (c) and (e) are known to be bactericidal and the ones on the right are known to be bacteriostatic antibiotics (b), (d), and (f) for the bacteria used (Only selected concentrations of antibiotics shown).

As seen in figure 2, the software also reports best estimates of all the above quantities. This enables us to independently estimate factors contributing to the measured impedance: the electrode capacitance (C_e), the electrode interface resistance (R_e), the bulk solution resistance (R_b) (inverse of solution conductance) and the bulk capacitance (C_b). This allows us to isolate not only the effects of temperature fluctuations that affect solution conductance (and hence R_b), but also electrode corrosion that affects R_e and C_e . The primary contributor to the bulk capacitance is the cell-membrane polarization, the effect of minor (<25°C) temperature fluctuations on which is known to be negligible [31].

As seen from the two individual fits in figure 2, which show the same suspension of *E. coli* in 8mg/ml Ampicillin at two different points in time (0 hrs and 2 hrs), the values of R_b , C_b , C_e , and L_e have not changed significantly, whereas the C_b has dropped significantly, presumably due to the loss of membrane integrity of some of the bacterial cells. That the value of R_b is relatively unaffected despite the potential release of intracellular content into the suspension and release of metabolites by living cells, is also to be expected. The volume of the 5×10^5 bacterial cells present per ml, each ~ 1 micron in diameter, is $\sim 10^{-7}$ ml, and hence any release of intracellular contents is unlikely to affect the bulk solution conductance (resistance) significantly. Similarly given the low rates of bacterial metabolism [consumption of 2×10^{-14} moles of O_2 /hr per individual bacterium [18], bacterial metabolism also does not contribute significantly to change in resistance unless the concentration unless their concentration is $\sim 10^8$ /ml [32].

The values of C_b calculated at each point in time are recorded. Deviations from their individual values at time $t=0$ are plotted in the figure 3.

Results and Discussion

In figure 3, we show (for selected samples) how the C_b changes over time for various suspensions. Since the C_b at time $t=0$ is different for individual samples (due to differences in the exact number of live bacteria present), we plot the difference from the initial value [$\Delta X_b(\tau) = X_b(\tau) - X_b(\tau=0)$] to aid in visualization of the changes. For all bacteria-antibiotic pairs, the C_b values increase monotonically with time when the concentration of the antibiotic is less than the MIC. At or above the MIC, however, the C_b values either begin to fall (ΔC_b negative), or remain unchanged ($\Delta X_b \approx 0$), depending on whether the antibiotic exerts a bactericidal or bacteriostatic effect, respectively. In some cases, as in the data shown in figures 3a and 3b (*E. coli*), the differences between growth, stasis, and cell-death are clearly discernible from the plot. In some other cases, such as in Figures 3e and 3f (*S. aureus*), the differences are not as obvious.

To draw more objective inferences from the data, the recorded values of C_b at different points in time (t) are fit to the mathematical model mentioned in equation (2) using the software R™. The output from the software gives its best estimate for variables in the equation (2), including that for K (Specific growth rate) and the “P-value” for the estimate. These values are displayed in table 1 for all antibiotic-bacteria pairs tested.

The K -value indicates whether the bacteria in the sample are proliferating ($K > 0$), dying ($K < 0$), or static ($K = 0$). The accompanying P-value indicates the probability that the true value of K is zero, and has a value between 0 and 1. If the P value is greater than 0.9 for a given sample set, then the value of K is considered to be zero. Thus, a sample with a positive K and P value < 0.9 has bacteria whose numbers

are exponentially increasing, while those with a negative K and a P-value of <0.9 has bacteria that are being killed (Bactericidal effect). However, irrespective of the value of K, if the P-value is >0.9, this is taken to indicate that there is no change in the number of live bacteria in the sample (Bacteriostatic effect). Using this criterion to determine growth, death or stasis, the minimum concentration of antibiotic, for which the value of K becomes statistically zero (or clearly negative) is the MIC. As listed in table 2, in all cases, MIC values obtained using our method fall within the expected range for the chosen antibiotic-bacteria combination. Also, for systems where the action of the antibiotic is known to be bacteriostatic (Amikacin for *P. aeruginosa*; Chloramphenicol for *E. coli* and *S. aureus*), the P-value remains >0.9

for all concentrations of antibiotic greater than the MIC. In contrast, at concentrations >MIC of the known bactericidal drugs (Ampicillin and Gentamicin), not only is the value of K negative, but the P-value is <0.9, indicating cell die-off.

Conclusion

To conclude, we would like to highlight that in this work, we have demonstrated the feasibility of using our novel electrical method to not only determine the MIC of candidate antibiotics to bacterial strains of interest in 4 hours, but also to infer the effect of the antibiotic on the strain (whether bactericidal or bacteriostatic) simultaneously. A major drawback of the technique (as it currently stands) is that it is quite labor-intensive, making it not well-suited for use in the clinic/point-of-care. However, efforts are currently underway to automate the aliquot collection, impedance measurement and data analysis. It will also require extensive tests against a wider panel of antibiotics and strains before it can be actually tested in a clinical setting. In fact, once automated and made user-friendly, the described method can also be extended to obtain other relevant clinical information as well. For instance, by obtaining electrical measurements at more frequent intervals, it could become possible to conduct time-kill studies in real-time, thereby indicating which antibiotic is able to kill the infectious bacteria faster. Thus, the work reported here represents “proof-of-principle” for a method that can, in the future, be used to rapidly obtain not only MICs, but also other information regarding the antibacterial mode of action.

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Antibiotic Concentration (mg/l)	Value of K from eqn.(2) calculated using RTM software, with P-value in parentheses					
	<i>Escherichia coli</i> ATCC 25922		<i>Pseudomonas aeruginosa</i> ATCC 27853		<i>Staphylococcus aureus</i> ATCC 29213	
	Ampicillin	Chloramphenicol	Gentamicin	Amikacin	Ampicillin	Chloramphenicol
Control	0.53876 (0.035)	0.4479 (0.6735)	0.04821 (0.819)	0.13 (0.756)	0.03685 (0.839)	0.2772 (0.0648)
0.5	0.692 (0.368)	0.56079 (0.856)	0.06857 (0.819)	1.73 (0.43)	-0.026 (0.878)	0.4332 (0.849)
1	0.59 (0.368)	0.2664 (0.75)	0.5607 (0.639)	0.2525 (0.539)	-0.4457 (0.1673)	1.42 (0.707)
2	-0.278 (0.75)	0.0806 (0.794)	0.15 (0.971)	0.792 (0.62)	-0.53 (0.26)	0.671 (0.96)
4	-0.898 (0.2285)	0.227 (0.628)	-0.7194 (0.55)	-0.0013 (1)	-0.3969 (0.415)	0.0268 (0.95)
8	-1.23 (0.36)	0.209 (0.941)	-0.6121 (0.7918)	-0.7373 (0.995)	-0.568 (0.191)	-0.632 (0.944)
16	-0.511 (0.66)	-0.04 (0.945)	-1.0139 (0.495)	-0.065 (0.995)	-0.2478 (0.609)	-0.1003 (0.94)
32	-3.54 (0.064)	-0.0089 (0.989)	-0.1791 (0.6486)	-0.0069 (0.90)	-0.4627 (0.5916)	0.05014 (0.967)
64	-10.35 (0.083)	-0.0119 (0.995)	-0.152 (0.827)	-0.38 (0.922)	-0.291 (0.174)	-0.1814 (0.96)
128	-2.35 (0.6083)	0.024 (0.994)	-0.5723 (0.1889)	-0.04121 (0.987)	-2.25 (0.189)	-0.02605 (0.982)

¹The values of K obtained by fitting the recorded values of bulk capacitance (C_b) at different points in time (t) to Equation (2), using the Software RTM (with P-values, also calculated by R, in parenthesis) for all samples tested (each with a different concentration of antibiotic). The boxes corresponding to the MIC value for each antibiotic-bacteria pair, as determined by criteria described in the text, is marked with thick borders. The shaded areas indicate the expected MIC range for a given antibiotic-bacteria pair.

Table 1: Value of K from equation (2) calculated using RTM software, with P-value in parentheses¹.

Bacteria	Antibiotic	Known MIC (mg/l)	Our MIC (mg/l)
<i>Escherichia coli</i> (ATCC 25922)	Ampicillin (Bactericidal)	2-8 [22]	2
	Chloramphenicol (Bacteriostatic)	2-8 [22]	8
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	Gentamicin (Bactericidal)	1-4 [24]	1
	Amikacin (Bacteriostatic)	2-8 [22,26]	4
<i>Staphylococcus aureus</i> (ATCC 29213)	Ampicillin (Bactericidal)	0.5-2 [23]	0.5
	Chloramphenicol (Bacteriostatic)	2-8 [25]	2

Table 2: Comparison of MIC values obtained using our method to known MIC values for all combinations of antibiotic and bacterial strains tested.

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