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ACS Appl. Mater. Interfaces, **Just Accepted Manuscript** • DOI: 10.1021/acsami.6b16571 • Publication Date (Web): 14 Mar 2017

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Rapid Real-time Antimicrobial Susceptibility Testing with Electrical Sensing on Plastic Microchips with Printed Electrodes

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KEYWORDS Antibiotic Susceptibility test, Electrical sensing, Antibiotic resistant pathogen, Flexible electronics, Screen-printed electrodes

ABSTRACT: Rapid antimicrobial susceptibility testing is important for efficient and timely therapeutic decision making. Due to globally spread bacterial resistance, the efficacy of antibiotics is increasingly being impeded. Conventional antibiotic tests rely on bacterial culture, which is time-consuming and can lead to potentially inappropriate antibiotic prescription and up-front broad range of antibiotic use. There is an urgent need to develop point-of-care platform technologies to rapidly detect pathogens, identify the right antibiotics, and monitor mutations to help adjust therapy. Here, we report a biosensor for rapid (<90 min), real time, and label-free bacteria isolation from whole blood and antibiotic susceptibility testing. Target bacteria are captured on flexible plastic-based microchips with printed electrodes using antibodies (30 min) and its electrical response is monitored in the presence and absence of antibiotics over an hour incubation time. We evaluated the microchip with *Escherichia coli* (*E. coli*) and methicillin-resistant *Staphylococcus aureus* (MRSA) as clinical models with ampicillin, ciprofloxacin, erythromycin, daptomycin, gentamicin, and methicillin antibiotics. The results are compared with the current standard methods, *i.e.* bacteria viability and conventional antibiotic assays. The technology presented here has the potential to provide precise and rapid bacteria screening and guidance in clinical therapies by identifying the correct antibiotics for pathogens.

Introduction

Rapid evolution of bacterial resistance toward antibiotics and the slowdown in progress of developing new antibiotics are critical threats to public health in the foreseeable future¹. Every year at least 2 million people in the United States acquire serious infections with bacteria that are resistant to one or more of the antibiotics designed to treat those infections. As a direct result of these antibiotic-resistant infections at least 23,000 Americans die each year². Current conventional antibiotic testing involves multiple bacterial culture steps such as disk diffusion, Luria-Bertani (LB) broth serial dilutions, and antimicrobial chemical reactions to detect bacteria and test its antibiotic susceptibility³. These assays provide vital information on the impact of antibiotic on bacteria and suitable guidance to select the proper antibiotic regimen but are labor-intensive and time-consuming (24 hours to 5 days)⁴⁻⁵. Patients are usually treated with empirical antibiotics to cover most

probable pathogens, which can result in incorrect initial therapy leading to further infection and increased mortality. Molecular diagnostic assays that target DNA and genetic mutations associated with drug-resistance require pre-knowledge with respect to the genetic basis of antibiotic resistance for a target pathogen⁶⁻¹¹. Polymerase chain reaction (PCR)-based assays have limitations, including complex nucleic acid amplification steps, false positive and negative results due to amplification of complex matrix media and enzymatic inhibition from blood and urine components¹²⁻¹³. Numerous other techniques have also been developed for antibiotic susceptibility testing such as nucleic acid staining⁸, nanoliter optical sensing of stochastic confinement¹⁴, electrochemical bacteria respiration¹⁵⁻²⁰, motion detector using atomic force microscopy²¹, optical detection of bacterial growth²²⁻²⁵, and mass monitoring of bacterial growth using cantilevers²⁶. Almost all of these techniques require bulky and complex equipment to perform sensing. The electrochemical sensing-based method requires time-

consuming redox concentration optimization for electrochemical respiration monitoring. Electrical sensing modality is a robust, label-free, rapid, and easy-to-use technique that has been employed for various bio-sensing applications such as bacteria detection²⁷ and monitoring of bacteria growth in various media²⁸⁻²⁹. The electronic circuit design for electrical sensing is simple. Here, we report a rapid real-time antimicrobial susceptibility testing using electrical sensing of captured bacteria on plastic microchips with printed electrodes in the presence and absence of antibiotics (Figure 1). Figures 1A,B show the schematic and actual photograph of the biosensor. The components of the microchip are shown in detail in Figure S1 (Supporting Information). Components of the microchip for antibiotic testing are: (A) plastic substrate, (B) screen-printed carbon electrodes on the back side of the plastic substrate for on-chip sample temperature control and sensing, (C) screen-printed silver interdigitated electrodes on the front side of the plastic substrate, and (D) attached DSA and PMMA sheets on the front side of the plastic substrate to create a closed microchip system. The presented method relies on the significant effect of antibiotics on the growth of captured bacteria on-chip. We first evaluated our hypothesis by monitoring the electrical response of bacteria in the presence and absence of antibiotics on non-functionalized chips (Figure 1C). Our results demonstrated that the electrical signal can significantly

change due to the presence of an effective antibiotics over a period of 1 h.

We evaluated the microchip using *E. coli* and MRSA as two clinical models. We also evaluated the ability of the microchip to selectively isolate MRSA from whole blood using antibody and tested its electrical response against gentamicin antibiotic (Figure 1D).

2. Materials and Methods

2.1 Materials and cell culture

LB agar, ampicillin (A9393), erythromycin (E6376), ciprofloxacin (17850), methicillin (51387), daptomycin (D2446), gentamicin (G1914), Tris (2-carboxyethyl) phosphine and LB Agar plates (L5542) were purchased from Sigma Aldrich (St. Luis, MO). LB broth (BP09723) was bought from Fischer Scientific (Pittsburgh, PA). *Escherichia coli* K12 (ATCC[®] 700891™) and methicillin-resistant SRM551 were cultured on a shaker overnight (12 h) at 37 °C in 2% LB broth media. The bacteria pellet was gently separated using a centrifuge for 5 min at 3000 rpm and the pellet was re-suspended in fresh LB broth media. Concentrations of bacteria were set to a constant optical density (OD) value, which was measured by UV-vis from NanoDrop, ND-1000 (Thermo Scientific, Wilmington,

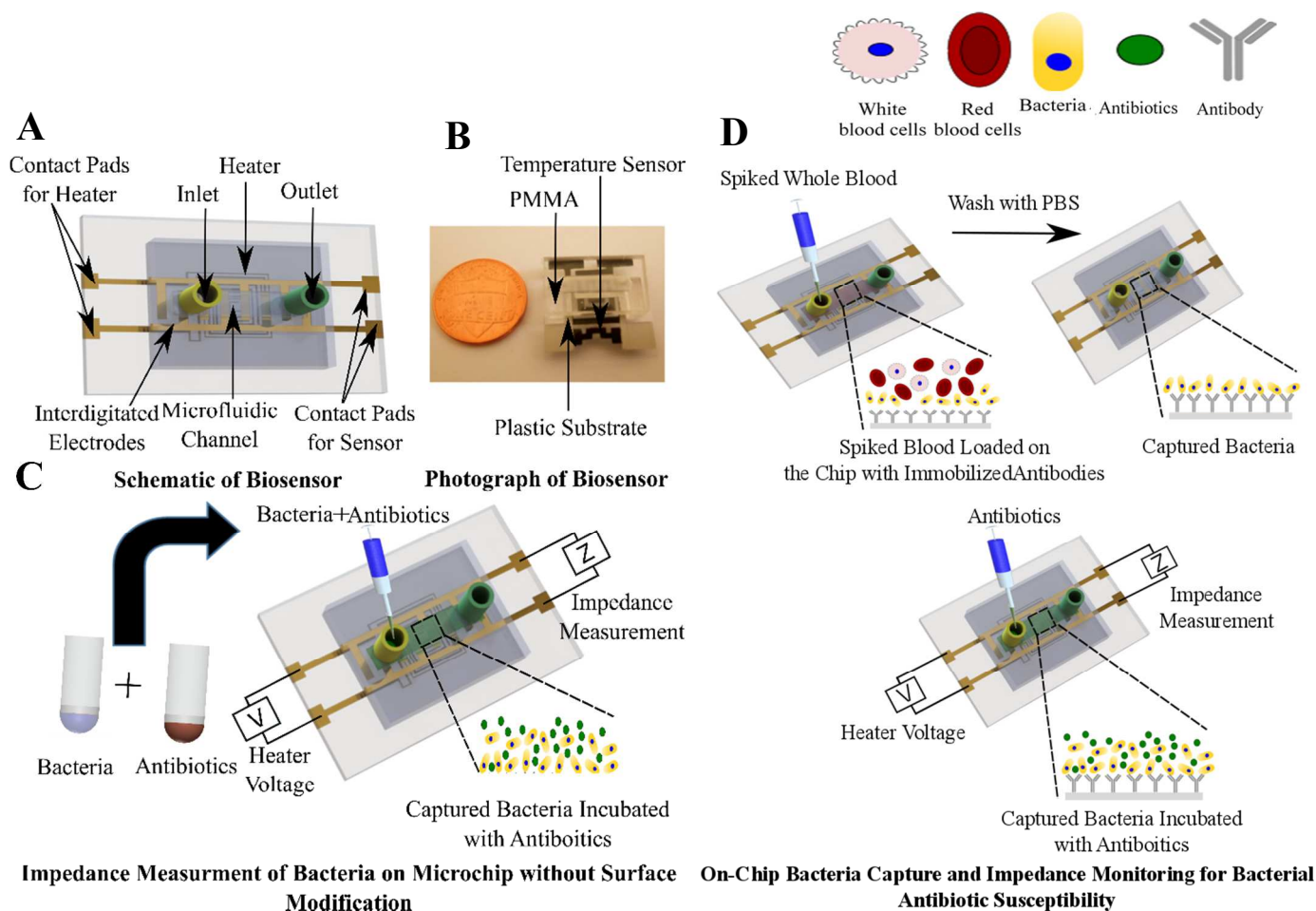


Figure 1. Experimental Design. (A) Schematic diagram of the biosensor with microfluidic channel, (B) Photograph of a biosensor with inter-digitated electrodes, PMMA microfluidic channel, integrated heater and temperature sensor, (C) Antibiotic susceptibility measurement using a label-free electrical sensing on biosensor without surface modification, (D) On-chip bacteria capture and isolation from whole blood using affinity based sensing and measurement of bacterial antibiotic susceptibility using electrical sensing.

DE). The OD of the samples (*E. coli* and MRSA in LB broth media) was set to 0.125, which corresponds to 10^8 CFU/ml.

2.2 Affinity-based bacteria capturing

An affinity-based approach was used to confirm specific and efficient capturing of MRSA on-chip. Anti-MRSA antibody (ab62742, abcam, Cambridge, MA) with 10 μ g/ml concentration was injected into the microchannel (30 μ l) and incubated for 30 min. The channel was then washed three times using 30 μ l $1 \times$ PBS. Then, 30 μ l of MRSA-spiked whole blood was added into the chip and incubated for 30 min to allow bacteria capture on-chip. The microchannel was then washed three times using 30 μ l PBS.

2.3 Antibiotic susceptibility assay viability

Cultured MRSA and *E. coli* were diluted to 10^3 CFU/ μ L and incubated with different antibiotics of clinically relevant concentrations ranging from 0.1 μ g/ml to 100 μ g/ml.⁴ The optical density (OD) value at 600 nm was measured using plate-reader Synergy2 (BioTek, Winooski, VT). After 24 hours, the OD was measured and viability of the bacteria was calculated based on differences between the ODs before and after incubation⁴.

2.4 Impedance analyzer

The impedance of the bacterial pathogen (with and without antibiotics) placed on the interdigitated electrodes was measured using an impedance analyzer (LCR8110G, GW Instek, CA) at 1 V for frequencies between 100 Hz and 1 MHz. From our previous studies^{9, 30-31}, we have found that the change in the impedance is maximum at 1 kHz. Additionally, we found a high signal-to-noise ratio for 1 kHz. Thus, we have selected 1 kHz as the frequency to study the response of bacterial pathogens. For on-chip antibiotic susceptibility utilizing impedance monitoring, the impedance of the bacteria sample in presence and absence of antibiotics was monitored at 1 kHz for 60 min with scanning interval of 10 min and it was normalized with respect to the initial impedance magnitude ($t=0$).

(VLS 2.3 laser printer, Universal Laser System Inc.). The size of the device was 20 mm $\times$ 20 mm (length $\times$ width). The active area of the device was 10 mm $\times$ 4 mm (length $\times$ width) with pattern interdigitated electrodes (0.6 mm width and 0.8 mm spacing). The schematic diagram of the heater and biosensor are shown in Figures 1A,B. The screen was designed using CorelDRAW[®] and windows were created for IDEs and heater on the DSA film using a laser cutter. 100 μ m Clear flexible plastic sheets (3M, Maplewood, Minnesota, USA) were used as substrate for electrode printing. On one side of the substrate, the screen for IDEs was fixed and silver (Engineered Materials System, INC, <0.015 Ω /m) and silicone (DAP Products Inc, Baltimore, MD) paste (4:1) was screen printed to form the IDEs. The plastic substrate with IDEs was dried at 90 $^{\circ}$ C for 30 minutes. On the other side of the substrate, a heater and temperature sensor were developed in the same way using a low resistive conducting carbon ink (<10 Ω /m, Engineered Materials System, INC) (Figure 1B). The channel dimensions for loading the biological fluid with the bacterial pathogen was 13.85 mm $\times$ 3.43 mm $\times$ 0.7 mm (length $\times$ width $\times$ depth). To create the microfluidic channel, one of the protective layers of DSA was peeled off and attached to PMMA. PMMA/DSA sheets were cut using a laser cutter with power, scan speed, and pulse per inch rate of 24 W, 5 mm/s, and 1000 pulses/inch, respectively. The protective layer from one side of DSA was peeled off before attaching it to the sensor. The chip reservoir was covered with a plastic tape during performing the experiments.

2.6 Statistical analysis

Statistical analysis on all results was performed using one-way ANOVA with the test for single column comparison with respect to the control sample. Statistical significance threshold was set at 0.05 ($p < 0.05$). Error bars represent a standard deviation of the mean ($n=3$). Asterisk shows statistically significant impedance magnitude change compared to all other data sets.

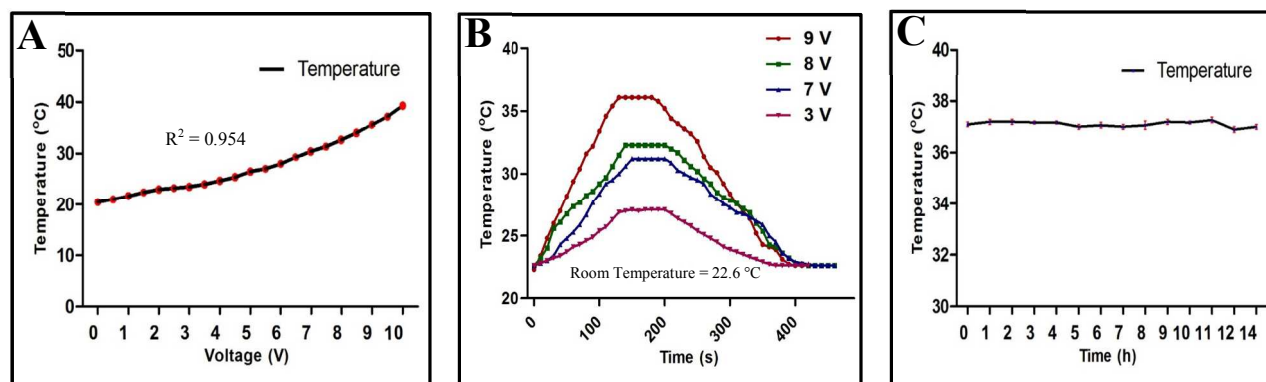


Figure 2. Electrical characterization of a screen printed heater on a flexible plastic substrate. (A) Plot shows a linear relationship between applied voltage and temperature with R^2 of 0.954 in an empty microchannel, (B) Shows the response of the heater for different voltages, and (C) Temperature stability (37 $^{\circ}$ C) of the heater for 14 h at constant voltage of 9V in an empty channel. Error bars represent standard error of the mean ($n=3$), $p<0.005$.

2.5 Biosensor fabrication on a plastic substrate

A microchip involving interdigitated electrodes (IDEs), a heater, a temperature sensor, and PMMA/DSA (polymethyl methacrylate/double sided adhesive film) microfluidic channels was fabricated using screen-printing³² and laser cutting

3. Results and Discussion

3.1 On-chip microheater

In order to achieve 37 $^{\circ}$ C for on-chip bacteria incubation, the electrical characterization of the screen-printed heater was performed (Figure 2). We observed a linear response for

change in temperature of the heater with respect to increase in voltage ($R^2=0.95$) (Figure 2A). The change in resistance was converted to temperature, which was measured by an on-chip temperature sensor. The heater required 9 V to reach a stable temperature of 37 °C. To evaluate the response of the heater, a constant voltage was applied until the heater's temperature reached to an asymptotic value. After waiting for 60 s, the voltage was brought down to 0 V, which resulted in the heater reaching room temperature (Figure 2B). We observed that the turn on and off times for the heater to reach 37 °C and room temperature (21 °C) was 100 s and 200 s, respectively. The heater was stable at 37 °C for 14 h at 9 V (Figure 2C) ($n=3$). The microchannels were empty during these experiments.

3.2 Electrical response of bacteria on-chip in the presence and absence of antibiotics

The electrical response of the bacteria in the presence and absence of antibiotic was first evaluated using non-functionalized chips. In these proof-of-concept experiments,

we wanted to monitor the electrical response of the bacteria in the presence and absence of antibiotics over time without considering the complexity of on-chip bacteria capture and isolation. Therefore, bacteria-spiked LB broth samples were injected into non-functionalized chips and its impedance response was monitored in the presence and absence of antibiotics. The antibiotic susceptibility assay was performed using six antibiotics: ampicillin, ciprofloxacin, and erythromycin on *E. coli* and daptomycin, gentamicin, and methicillin on MRSA. For Urinary Tract Infections (UTI), the concentration of bacteria $>10^5$ CFU/ml is considered clinically relevant⁵. The impedance magnitudes of samples (30 μ L), at any time during the experiments were normalized with respect to the initial impedance magnitudes of the samples ($t=0$). In order to be certain that the antibiotic is fully effective we selected higher concentration of the bacteria (10^6 CFU/mL). Figures 3A, E, I show the real-time change in impedance magnitude from $t=0$ to $t=60$ minutes at 1 kHz and 1 V. We observed significant difference between the electrical response of spiked and con-

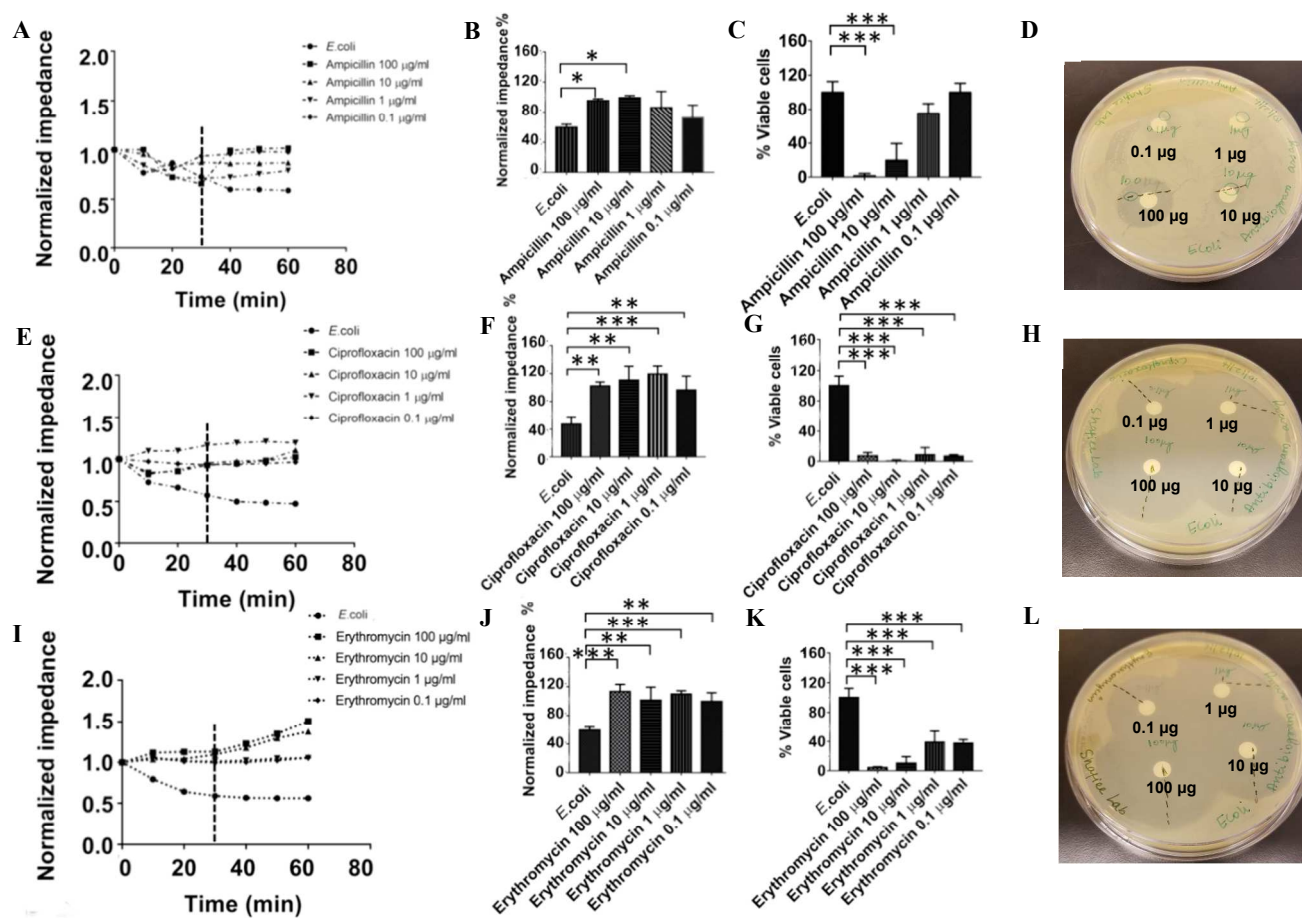


Figure 3. Electrical response of *E. coli* in the presence of Ampicillin, Ciprofloxacin, and Erythromycin over a period of 1 h. *E. coli* in the presence of Ampicillin, Ciprofloxacin, and Erythromycin over a period of 1 h. (A) Real time impedance measurement of *E. coli* in presence of ampicillin, (B) Statistical analysis of the effect of ampicillin on *E. coli* ($p=0.0055$, $n=3$), (C) Viability test results of *E. coli* in the presence of ampicillin after an overnight incubation by measuring their optical density (OD) at 600 nm wavelength, ($p<0.0001$, $n=3$), (E) Real time impedance measurement of *E. coli* in presence of ciprofloxacin, (F) Statistical analysis of the effect of ciprofloxacin on *E. coli* ($p=0.1915$, ns, $n=3$), (G) Viability test results of *E. coli* in the presence of ciprofloxacin ($p=0.0850$, $n=3$), (I) Real time impedance measurement of *E. coli* in presence of erythromycin, (J) Statistical analysis of the effect of erythromycin on *E. coli* ($p=0.0055$, $n=3$), (K) Viability test results of *E. coli* in the presence of erythromycin ($p=0.0850$, $n=3$), and (D, H, L) shows the antibiotic susceptibility of *E. coli* against ampicillin, ciprofloxacin, and erythromycin using disc diffusion method (24 h).

trol samples at t=30 min and afterwards. We chose t=30 min for statistical analysis between the normalized impedance magnitudes because we intended to develop a rapid diagnostics for antimicrobial susceptibility testing. Figures 3B, F, J show the normalized impedance magnitude of *E. coli*-spiked samples at t=30 minutes when ampicillin, ciprofloxacin, and erythromycin were used, respectively. The control samples were antibiotics-free *E. coli*-spiked samples (10^6 CFU/ml). These results showed that the normalized impedance magnitude of *E. coli*-spiked bacteria samples were significantly different than control samples at ampicillin concentrations of 10 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$ (Figure 3B, n=3, $p<0.001$) while, 1 $\mu\text{g/ml}$ and 0.1 $\mu\text{g/ml}$ did not show any statistical significance with respect to control (Figure 3B, n=3, $p>0.05$). The cell viabilities in the presence of 100 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$, 1 $\mu\text{g/ml}$, and 0.1 $\mu\text{g/ml}$ ampicillin concentration were $4.5\%\pm 1$, $20\%\pm 17$, $40\%\pm 8$, and $95\%\pm 4$, respectively which was significantly different than the cell viability of control samples (Figure 3C, $p<0.001$, n=3) and is in agreement with minimum

inhibitory growth (MIG) of ampicillin⁴. The normalized impedance magnitudes of *E. coli*-spiked samples, when ciprofloxacin at concentrations ranging from 0.1 $\mu\text{g/ml}$ to 100 $\mu\text{g/ml}$ was used, were significantly different than control samples (Figure 3E, F, n=3, $p<0.001$). The cell viability in the presence of ciprofloxacin at concentrations of 100 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$, 1 $\mu\text{g/ml}$ and 0.1 $\mu\text{g/ml}$ were $7.4\%\pm 4.6$, $0.9\%\pm 0.9$, $8.9\%\pm 8.9$, $6.8\%\pm 2.2$, respectively, which was significantly different than the cell viability of control samples (Figure 3G, n=3, $p<0.001$)⁴. Similarly, we observed significant electrical response for *E. coli* samples in the presence of erythromycin with concentrations ranging from 0.1 $\mu\text{g/ml}$ to 100 $\mu\text{g/ml}$ compared to antibiotic-free control samples (Figure 3J, n=3, $p<0.001$). The real-time change in impedance magnitude of *E. coli* for erythromycin shows an increase in normalized impedance magnitude for 100 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$ which could be

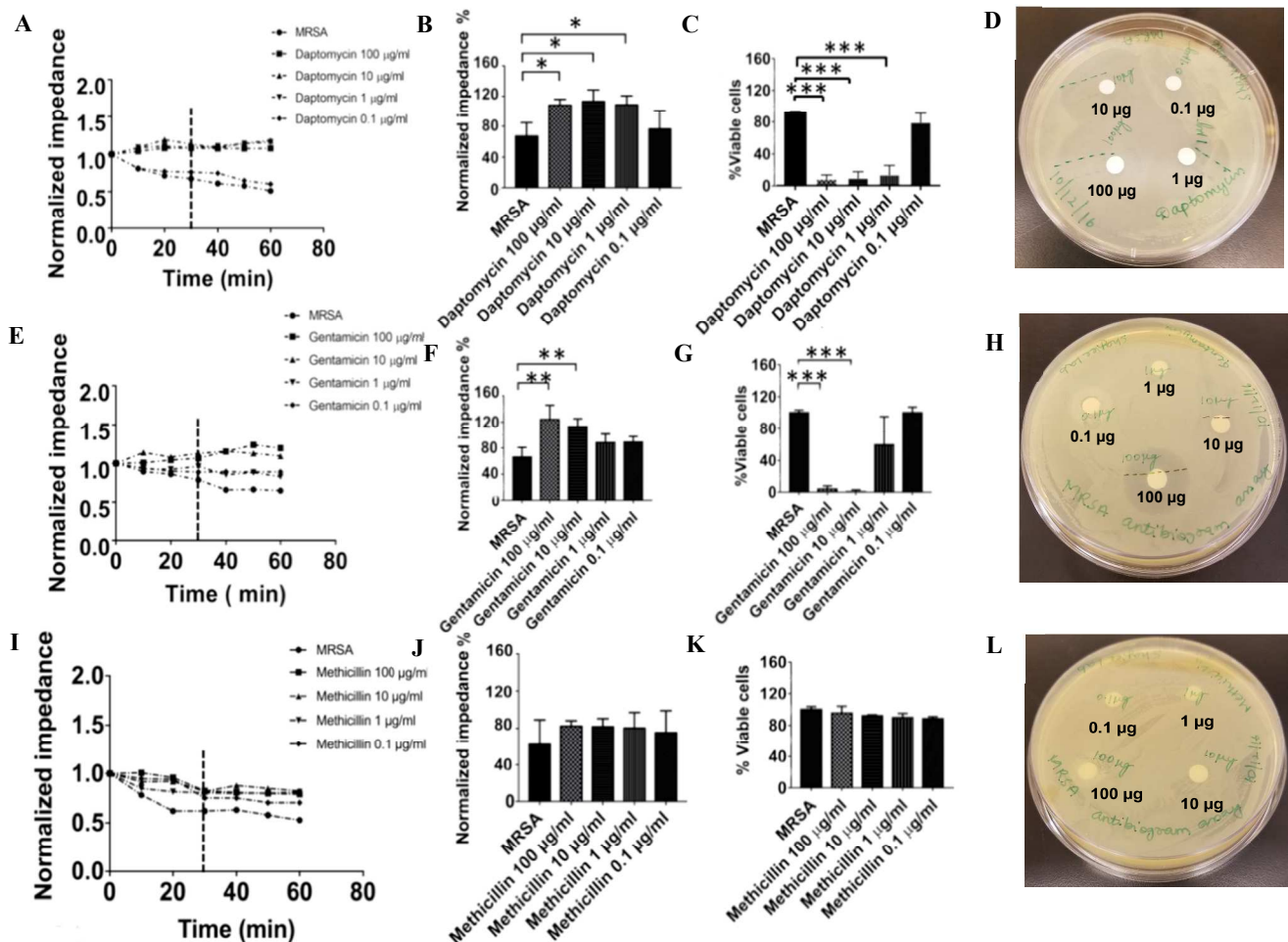


Figure 4. Electrical response of MRSA in the presence of Daptomycin, Gentamicin and Methicillin over a period of 1 h. (A) Real time impedance measurement of MRSA in presence of daptomycin, (B) Normalized impedance magnitude of MRSA-spiked samples in the presence of daptomycin at t=30 min ($p=0.05$, n=3), (C) Viability test results of MRSA in the presence of daptomycin after an overnight incubation by measuring their optical density (OD) at 600 nm wavelength, ($p<0.0001$, n=3), (E) Real time impedance measurement of MRSA in presence of gentamicin, (F) Normalized impedance magnitude of MRSA-spiked samples in the presence of gentamicin at t=30 min ($p=0.1915$, ns, n=3), (G) Viability test results of MRSA in the presence of gentamicin ($p=0.0850$, n=3), (I) Real time impedance measurement of MRSA in presence of methicillin, (J) Normalized impedance magnitude of MRSA-spiked samples in the presence of methicillin at t=30 min ($p=0.0055$, n=3), (K) Viability test results of MRSA in the presence of methicillin ($p=0.0850$, n=3), and (D, H, L) shows the antibiotic susceptibility of MRSA against daptomycin, gentamicin, and methicillin using disc diffusion method (24 h).

due to the low pH~6 of erythromycin that provides a higher positive charge of Erythromycin. The interaction between positively charged erythromycin with negatively charged bacteria cell membrane may neutralize the charge balance of the solution which consequently increases the impedance as shown in Figure 3I. The minimum inhibitory concentration (MIC) against erythromycin was less than 0.1 $\mu\text{g/mL}$, as *E. coli* viability in the presence of erythromycin with concentrations ranging from 0.1 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$ was negligible and significantly different from the viability of *E. coli* without erythromycin (Figure 3K, $n=3$, $p<0.001$). The viability of *E. coli* bacteria in the presence of erythromycin with concentra-

tions of 100 $\mu\text{g/mL}$, 10 $\mu\text{g/mL}$, 1 $\mu\text{g/mL}$, and 0.1 $\mu\text{g/mL}$ were $4.3\%\pm 1.1$, $10.3\%\pm 8.9$, $38.9\%\pm 15.1$, and $34\%\pm 5.3$, respectively (Figure 3K). The antibiotic susceptibility of *E. coli* against ampicillin, ciprofloxacin, and erythromycin using disc diffusion method (24 h) are in agreement with the cell viability and the electrical sensing results (Figures 3D, H, L). We also performed similar experiments with samples spiked with MRSA and evaluated its antibiotic susceptibility in the presence of daptomycin, gentamicin, and methicillin (Figure 4). By increasing the antibiotic concentration in the pathogen sample, the rate of bacteria production decreases and therefore there will be less charge in the sample solution. The reduction of the charge in the sample yields to small change in normalized impedance of samples with antibiotics compared with samples without antibiotics within the culturing time as shown in Figures 4A, E, I. The impedance magnitude of the MRSA-spiked samples in the presence of daptomycin with the concentration of 0.1 $\mu\text{g/mL}$ was not significantly different than control samples (Figure 4B, $n=3$, $p>0.05$), however, the impedance magnitudes of the MRSA-spiked samples in the presence of daptomycin with the concentrations of 1 $\mu\text{g/mL}$, 10 $\mu\text{g/mL}$, and 100 $\mu\text{g/mL}$ were significantly different than control at $t=30$ minutes (Figure 4B, $n=3$, $p<0.05$). The cell viability of MRSA in the presence of 100 $\mu\text{g/mL}$, 10 $\mu\text{g/mL}$, and 1 $\mu\text{g/mL}$ daptomycin were $8.7\%\pm 5.6$, $10.2\%\pm 8.5$, $14.7\%\pm 12.5$, respectively which were significantly different than the viability of MRSA-spiked samples with 0.1 $\mu\text{g/mL}$ daptomycin concentration and control samples (Figure 4C, $n=3$, $p<0.001$). The impedance magnitudes of the MRSA-spiked samples in the presence of gentamicin with the concentrations of 0.1 $\mu\text{g/mL}$ and 10 $\mu\text{g/mL}$ were not significantly different than control samples (Figure 4F, $n=3$, $p>0.05$). The cell viability of MRSA in the presence of 1 $\mu\text{g/mL}$ and 0.1 $\mu\text{g/mL}$ gentamicin were $60\%\pm 13$ and $82.7\%\pm 4.9$, respectively, which reflect the fact that gentamicin is not effective for concentrations below 10 $\mu\text{g/mL}$ (Figure 4G). These results also showed that the impedance magnitudes of MRSA-spiked samples in the presence of methicillin with concentrations ranging from 0.1 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$ were not significantly different than control samples (Figure 4J, $n=3$, $p>0.05$). The cell viability of MRSA-spiked samples in the presence of 100 $\mu\text{g/mL}$, 10 $\mu\text{g/mL}$, 1 $\mu\text{g/mL}$, and 0.1 $\mu\text{g/mL}$ of methicillin were $95.7\%\pm 4.2$, $93.2\%\pm 1.7$, $92.9\%\pm 3.6$, and $92.5\%\pm 0.5$, respectively, which were not significantly different than antibiotic-free control samples (Figure 4K, $n=3$, $p>0.05$). This observation may be attributed to the fact that the methicillin MIC is >400 $\mu\text{g/mL}$ for MRSA SRM551 samples³³. These electrical sensing read outs and cell viability results were in agreement with the disc diffusion-based results (Figures 4D, H, L).

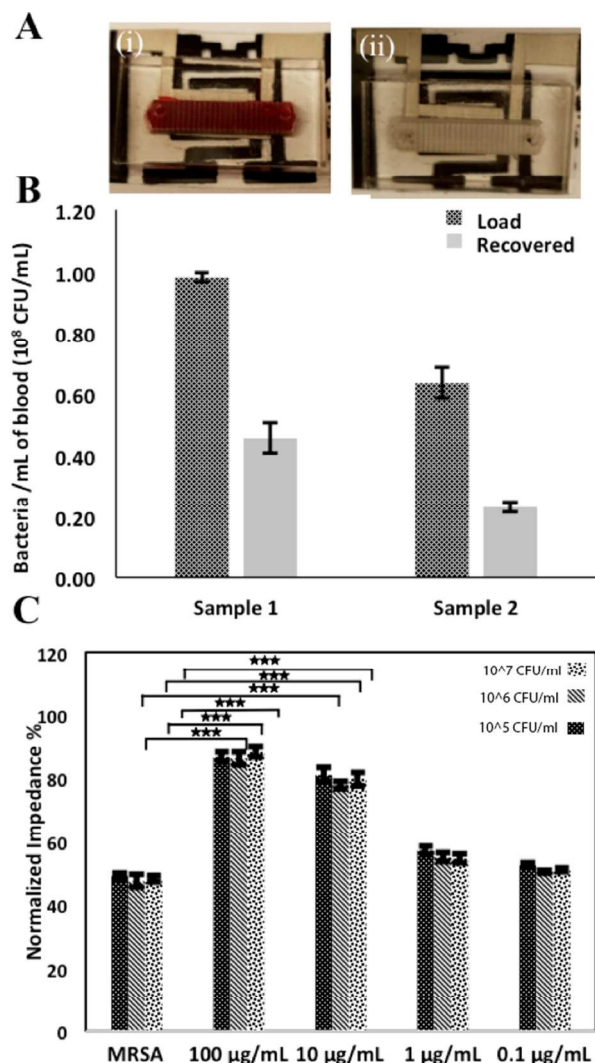


Figure 5. On-chip bacteria capture and antibiotic susceptibility testing. (A) Photograph of the fabricated microchip: (i) surface modified microchip loaded with MRSA-spiked whole blood (10^6 CFU/mL) (incubated for 30 min), (ii) Microchip washed with PBS, (B) Histogram plot indicating the bacterial load and bacteria recovery ($>43\%$) for the two spiked blood samples used in the study (sample 1: 10^8 CFU/mL and sample 2: 10^7 CFU/mL) ($n=3$). 10 $\mu\text{g/mL}$ of Anti-MRSA antibody was used for chip functionalization (C) Electrical response of the captured MRSA ($10^5 - 10^7$ CFU/mL) in the presence of Gentamicin (0.1 $\mu\text{g/mL}$ -100 $\mu\text{g/mL}$). Error bars represents standard error of mean ($n=3$). MRSA-spiked whole blood without antibiotic was used as control.

3.3 On-chip bacteria capture and antibiotic susceptibility testing

We also evaluated the ability of the microchip to selectively capture and isolate bacteria from whole blood and measured its electrical response in the presence of antibiotics. We selected MRSA-spiked whole blood samples with bacteria concentrations of 10^5 , 10^6 , and 10^7 CFU/mL. Figure 5A shows the actual photograph of the surface-modified microchip with immobilized anti-MRSA antibody loaded with MRSA-spiked whole blood (i) and the actual device after washing with PBS (30 μL) for three times (ii). Figure 5B shows bacteria recovery ($43\pm 2\%$) over bacterial loads (MRSA) (10^7 CFU/mL and 10^8 CFU/mL) spiked into 1 mL of whole blood. To calculate the

bacteria recovery rate using our functionalized microchips, we measured bacteria concentration in (i) MRSA-spiked whole blood sample used for this study and (ii) washed sample after releasing the captured bacteria on-chip by measuring the OD values of the sample. Captured bacteria were released after the washing step by cleaving the antibody using Tris (2-carboxyethyl) phosphine (TCEP) (10 $\mu\text{g/mL}$, 30 μL), a thiol-free compound that is highly effective at reducing protein and disulphide bond in antibody structure³⁴⁻³⁵. For performing on-chip bacteria capturing and antibiotic susceptibility testing, MRSA-spiked blood was loaded into the microchip and the electrical response of the captured bacteria was monitored at different concentration of gentamicin (Figure 5C). It should be noted that the electrical response of MRSA in non-functionalized microchips (Figure 4F) and microchips functionalized with anti-MRSA antibody (Figure 5C) demonstrated similar conclusion that 100 $\mu\text{g/mL}$ and 10 $\mu\text{g/mL}$ gentamicin concentration significantly affected MRSA viability and its electrical response compared to antibiotic-free MRSA-spiked samples. A same approach can be implemented for efficient capturing of *E. coli* in urine.

These results demonstrated the ability of the microchip to detect antibiotic susceptibility of bacteria spiked in LB broth and whole blood samples. This method can also be used for bacteria susceptibility testing in urine samples. We observed that the impedance magnitude changes of purified and unpurified urine samples spiked with *E. coli* and MRSA were lower compared to spiked LB broth and PBS samples (Figure 6, $n=3$, $P<0.001$). The normalized impedance magnitude of *E. coli*-spiked samples bacteria was $70\%\pm 2$ in LB broth, $30\%\pm 3$ in PBS, $16\%\pm 8$ in unpurified urine, and $15\%\pm 8$ in purified urine (Figure 6A). The normalized impedance magnitude of MRSA-spiked samples was $58\%\pm 7$ in LB broth, $49\%\pm 1$ in PBS, $37\%\pm 6$ in unpurified urine, and $22\%\pm 4$ in purified urine (Figure 6B).

We also developed a portable impedance analyzer as shown in Figure S2A. Figures S2B, C, D show the electronic module with a chip interfacing area, a sensor with silver interdigitated electrodes, a screen-printed carbon heater, and a conceptual plot showing the effect of antibiotics on the bacteria respectively (Supporting Information). The block diagram and circuit of the portable impedance analyzer are shown in Figure S3A, B, respectively. The AD5933 is a high precision impedance converter IC integrated with on-board frequency generator, 12-bit Analog-to-Digital converter (ADC) and an on-board DSP engine. The on-board frequency generator allows an external complex impedance to be excited with a known frequency. (i) The sample in the microfluidic device was excited and the impedance magnitude was measured. The desired excitation frequency or a series of excitation frequencies were programmed into the IC. The response signal was sampled by the on-board ADC and a discrete Fourier transform (DFT) was performed by the on-board DSP. The magnitude and relative phase of the impedance at each frequency point along the sweep were calculated, which was done off chip using the real and imaginary register contents, which was read from the serial I2C interface. (ii) PmodIA (Peripheral Module Impedance Analyzer) was interfaced with ATMEGA328 (a low-power CMOS 8-bit microcontroller). (iii) The ATmega328 was used to send commands and receive data from the PmodIA using I2C communication. The received data was sent to a PC by UART. (iv) To establish communication between the PC and ATmega328, a single-chip USB to UART bridge IC

(CP2102) was used. (v) We have developed a Python code that receives data at the corresponding COM port. The user can set up the initial parameters such as: starting frequency, a number of settling cycles, step size, a number of increments gain, etc. in the Graphical User Interface (GUI) of the Java program. The data was stored in a CSV file format for further analysis. Our portable impedance analyzer has the ability to plot the data by pressing the plot button in the GUI. We evaluated the performance of the portable impedance analyzer by measuring the data obtained from PBS solution (1%, 0.1%, and 0.05%), *E. coli*, and MRSA using portable impedance analyzer and comparing it with the data obtained from the

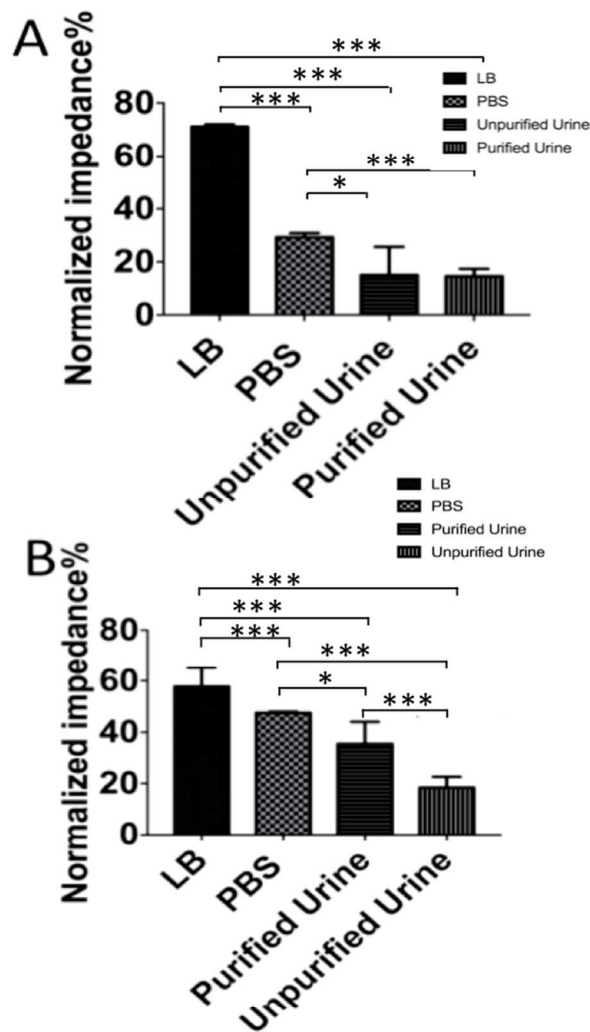


Figure 6. Monitoring the growth of microorganisms in different media by measuring their impedance magnitude over a period of 1 h. Electrical response of *E. coli* (A) and MRSA (B) during bacteria growth in LB broth, 1X PBS, and purified and unpurified urine.

impedance analyzer (Figure S4) (Supporting Information). In order to test the performance of the portable impedance analyzer we measured the data obtained from PBS solution (1%, 0.1%, and 0.05%), *E. coli*, and MRSA using portable impedance analyzer and compared with the impedance analyzer.

Conclusion

Here, we report a rapid (<90 min), label-free antimicrobial susceptibility testing through real-time monitoring of bacteria growth in the presence and absence of antibiotics. This device

can further be used to provide proper antibiotics in <90 min to patients suffering from UTI³⁶⁻³⁷ where the main causes are *E.coli* and *S.aureus* pathogens. The advantage of this technique over commonly used electrochemical monitoring for bacteria metabolic activity is that it does not require any redox molecule such as ferrocyanide or resazurin as well as redox concentration optimization, which significantly reduces the effort on assay optimization. We also performed an on-chip isolation of the target bacteria from the spiked whole blood with clinically relevant bacteria concentrations using antibody and monitored captured bacteria's electrical response in the presence and absence of antibiotics for antimicrobial susceptibility testing. We did not observe any sample evaporation-related issues in our study as the chip reservoirs were covered with plastic tapes during performing the experiments. In this study we chose *E. coli* and MRSA as two clinical models to evaluate the performance of the reported microchip technology. This electrical sensing-based mechanism for antibiotic testing can be potentially used for a broad range of bacteria including *Pseudomonas aeruginosa*³⁸, *vancomycin-resistant S. aureus* (VRSA)³⁹, *extended spectrum beta-lactamase* (ESBL), *vancomycin-resistant Enterococcus* (VRE) and multidrug-resistant *A. baumannii* (MRAB)⁴⁰⁻⁴¹. This method can be potentially used for detecting antimicrobial susceptibility testing for different strains of bacteria as long as there are different specific antibodies available for different bacteria strains. Further studies are needed to evaluate the specificity of electrical response of different strains of a bacterium in case there is a universal antibody is available for on-chip bacteria capture.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Microchip fabrication details, details of the portable impedance analyzer and its electronic circuit diagram, and evaluation of the portable impedance analyzer by comparing the results with the results obtained by an LCR meter.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

Research reported in this publication was partially supported by the National Institute of Allergy and Infectious Disease of the National Institute of Health under award number R01AI118502; Brigham and Women's Hospital and Harvard Medical School through Bright Futures Prize and Innovation Evergreen Award; National Engineering Research Council of Canada (NSERC) through NSERC Postdoctoral fellowship (M.S.). The authors also extend their gratitude to Karan Dhingra for performing preliminary experiments. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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