

Supporting Information for:

Rapid electrochemical phenotypic profiling of antibiotic-resistant bacteria

Justin D. Besant, Edward H. Sargent, and Shana O. Kelley

Electrochemical characterization of resazurin

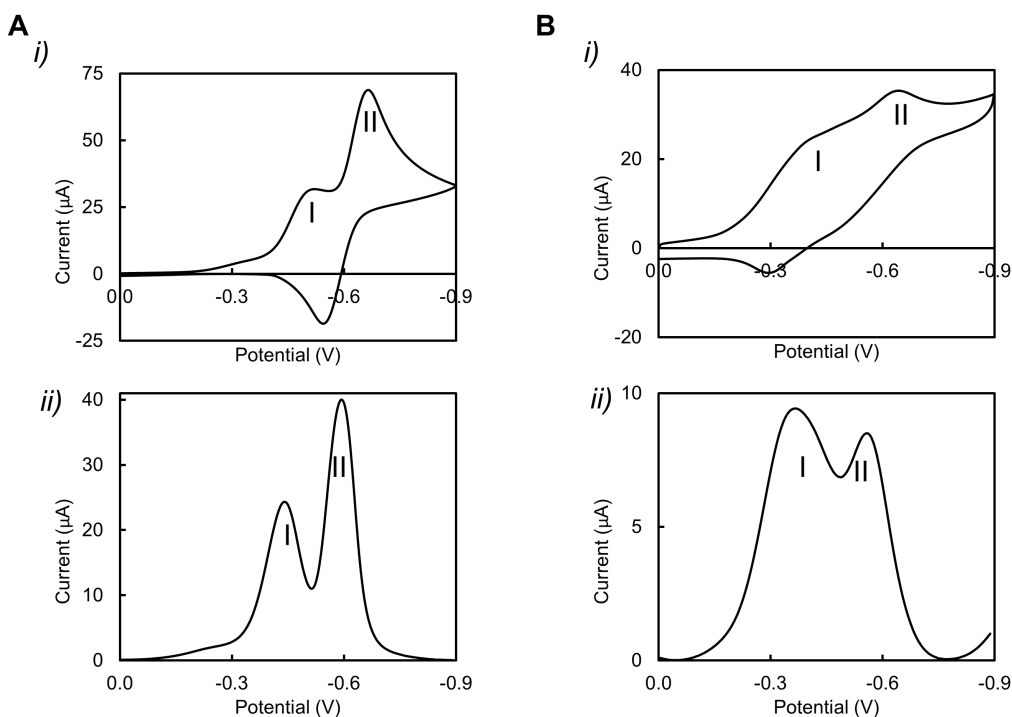


Figure S1. A) (i) Cyclic voltammograms and (ii) differential pulse voltammograms of 10 mM resazurin in PBS + 20% ACN. B) (i) Cyclic voltammograms and (ii) differential pulse voltammograms of 10 mM resazurin in LB media. Scans were acquired using an Au macroelectrode and a Ag/AgCl reference electrode.

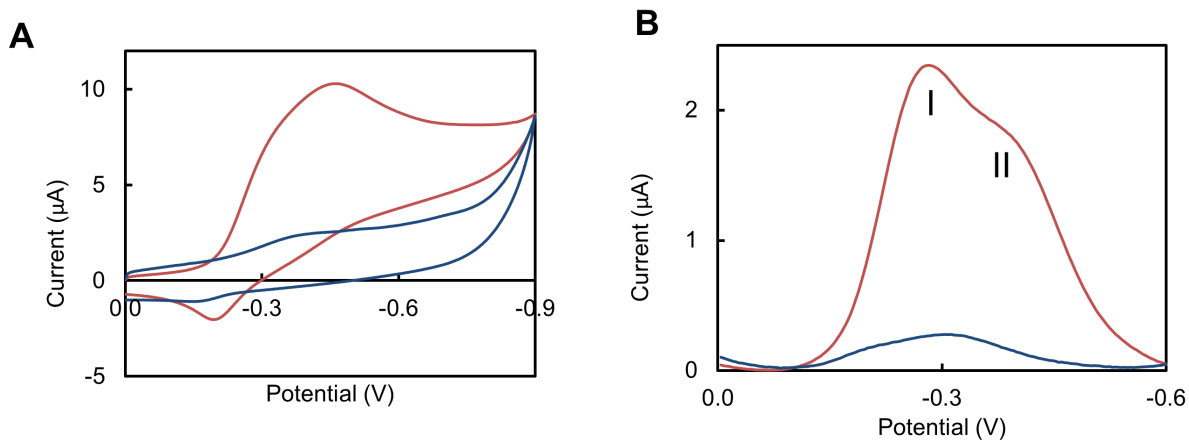


Figure S2. A) Cyclic voltammograms of *E. coli* at 1×10^6 CFU/mL before (red) and after (blue) incubating with 1 mM resazurin for 6 hrs at 37°C in LB media. B) Corresponding differential pulse voltammograms. Scans were acquired using an Au macroelectrode and a Ag/AgCl reference electrode.

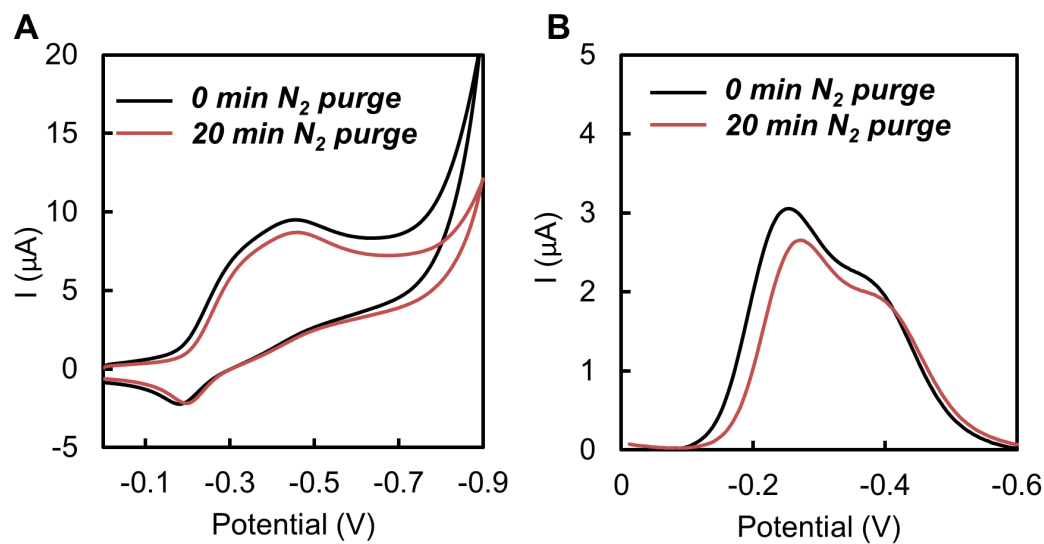


Figure S3. Effect of dissolved oxygen on the electrochemistry of resazurin. (A) CV and (B) DPV acquired using a gold macroelectrode with 1 mM resazurin in LB media before and after purging with N_2 for 20 min. We observed a 13% decrease in the DPV peak current after purging with N_2 .

Fluorescent detection of metabolically active *E. coli*

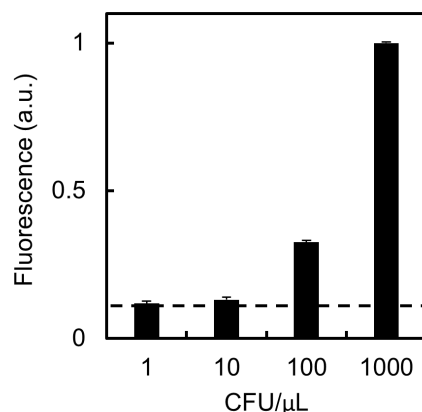


Figure S4. Fluorescent detection of metabolically active *E. coli*. Serial dilutions of *E. coli* were incubated for 5 hours at 37°C with 1 mM resazurin in LB media. The fluorescence signal was measured using a microplate reader at 585 nm with an excitation wavelength of 570 nm. Metabolically active bacteria convert resazurin to resorufin which increases the fluorescence signal. 100 CFU/μL were detectable using fluorescence which corresponds with the detection limit achieved using electrochemistry using the same 5 hr incubation period. The dashed line represents the signal from the blank sample.

Fabrication of the device

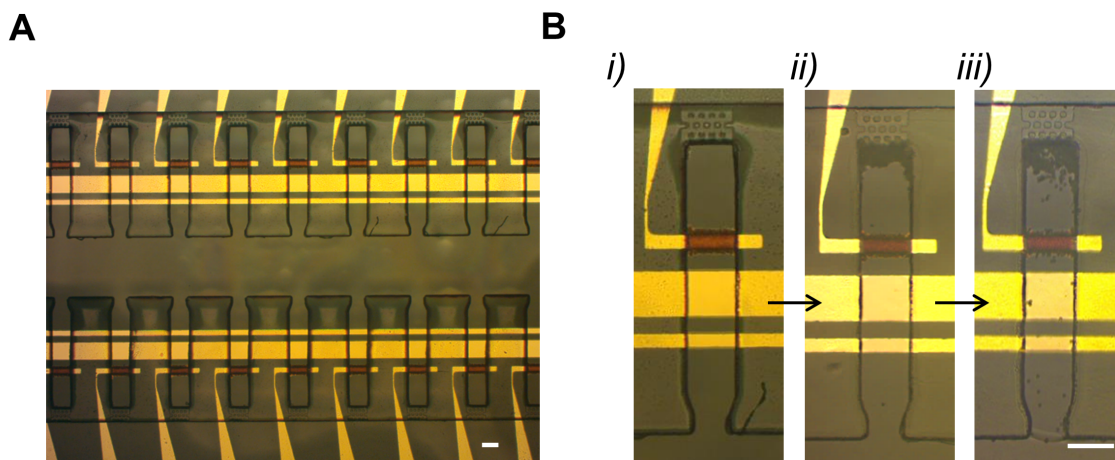


Figure S5. Optical images of the device. (A) An optical image of the well array. (B) (i) An optical image of a single well with a working, counter, and reference electrode before introducing the microbeads. (ii) Microbeads are trapped by a barrier at the rear of each well. (iii) After bacteria are captured, a nanoliter plug is formed by introducing an immiscible organic phase. Scale bars represent 100 μm.

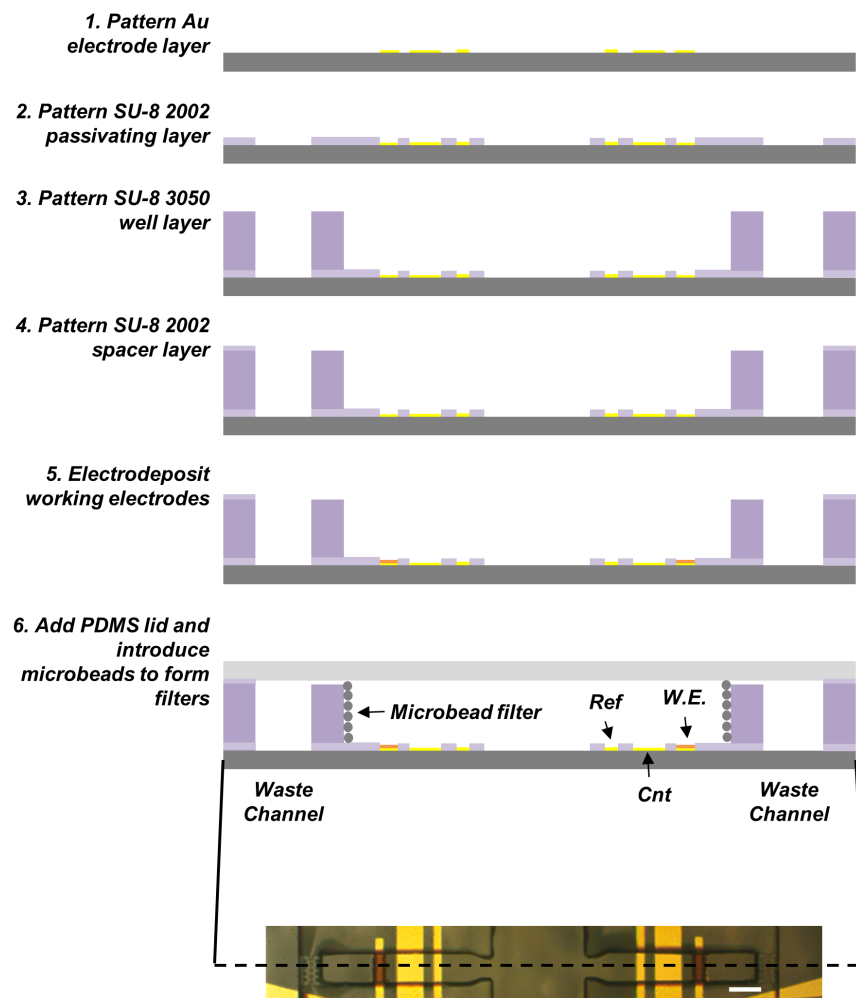


Figure S6. Steps in the fabrication of the device. (1) First a 100 nm gold layer is patterned using standard photolithography. Cr is used as an adhesion layer. (2) Next the patterned electrodes were passivated with 2 μm of SU-8 2002 using photolithography. (3) A 50 μm well layer was patterned using SU-8 3050. (4) A second layer of SU-8 2002 is patterned as a thin 2 μm spacer small enough to trap the 5 μm microbeads necessary to fabricate the in-well filters. (5) To increase the surface area of the electrodes, gold is electrodeposited on the working electrodes by applying -300 mV for 30 s with respect to an Ag/AgCl reference electrode in a solution of 50 mM HAuCl₄ and 0.5 M HCl. (6) The device is capped with a PDMS lid and 5 μm microbeads are injected into the wells to form the in-well filters.

Characterization of the in-well filter

Characterization of pore size. As the microbeads are spherical, we can refer to the extensive literature studying the packing of spherical objects. The densest possible packing of spheres is hexagonal close packing in which the packing fraction is 0.74 and the pore diameter is given by¹:

$$D_p = 0.154D_s$$

Where D_p is the diameter of the pores and D_s is the diameter of the spheres. For 5 μm diameter beads, assuming hexagonal close packing, the pore diameter is 0.77 μm which is sufficiently small to trap a bacterium ($\sim 1 \mu\text{m}$). In reality, the spheres would pack in an assembly close to random close packing which has a slightly looser packing with a packing fraction of 0.637, which causes a distribution in pore sizes, but does not change the diameter of the smallest pores.¹ This calculation is consistent with the high resolution images below. Using ImageJ, we measured the pore size and found a similar minimum pore size of approximately 0.8 μm .

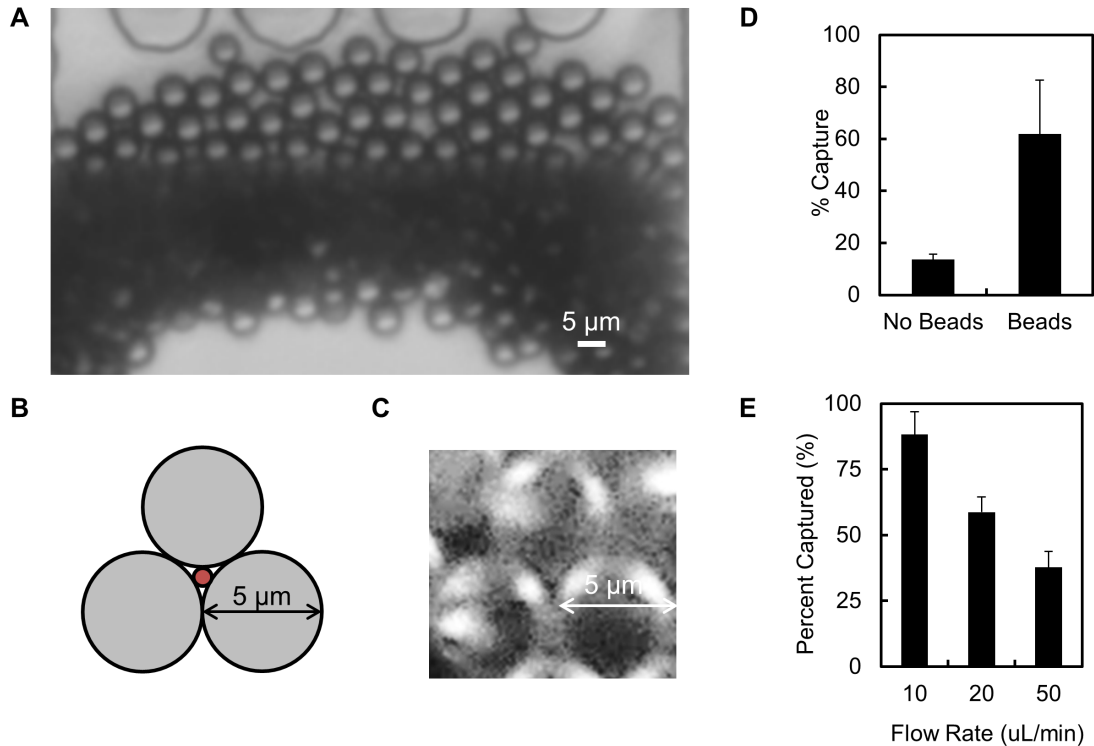


Figure S7. Characterization of the in-well filter. (A) Image of the filter bead bed acquired using optical microscopy. (B) Illustration showing the effect of bead size and packing on pore size. The diameter of the largest particle, represented in red, capable of fitting through spherical beads with hexagonal close packing is 0.77 μm . (C) Optical microscope image showing a close up of the beads. The pore size in this image is approximately 0.8 μm which is consistent with the calculations. (D) When microbeads are not used, bacteria are captured with low efficiency. (E) Capture efficiency of *E. coli* as a function of flow rate. Error bars represent standard error.

Measuring filter stability. To measure the stability of the microbead filters, we injected 100 μL of microbeads at 20 $\mu\text{L}/\text{min}$ into a version of the device without the in-well electrodes. We blocked the outer-channel inlet and the inner-channel outlet which forces the fluid through the wells. After stopping the flow, we acquired microscope images over the course of 1 hour. We found that the microbeads were stable over the course of 1 hour. Although a few beads did become dislodged from the filters, this does not affect the electrochemical measurements as the electrodes are offset by 200 μm from the filter.

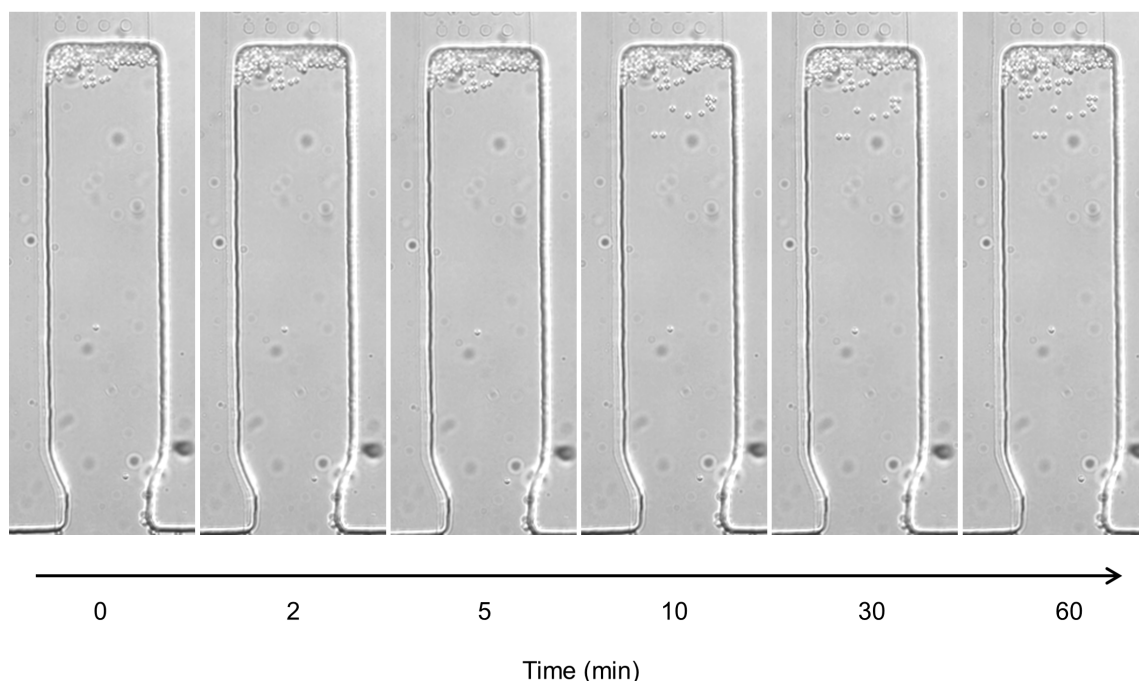


Figure S8. Characterization of filter stability. A series of optical images showing the stability of the filter over time.

Eluting bacteria from filters for capture efficiency measurements. To calculate the capture efficiency of the in-well filters, we eluted the captured bacteria and incubated off-chip on agar plates. To elute the bacteria, we inject buffer while directing the fluid flow backwards through the filters. This is accomplished by blocking the outer-channel inlet and the inner-channel outlet. The backflow of buffer forces bacteria out of the filters back towards the inlet. The eluent was cultured overnight at 37°C and the colonies were counted.

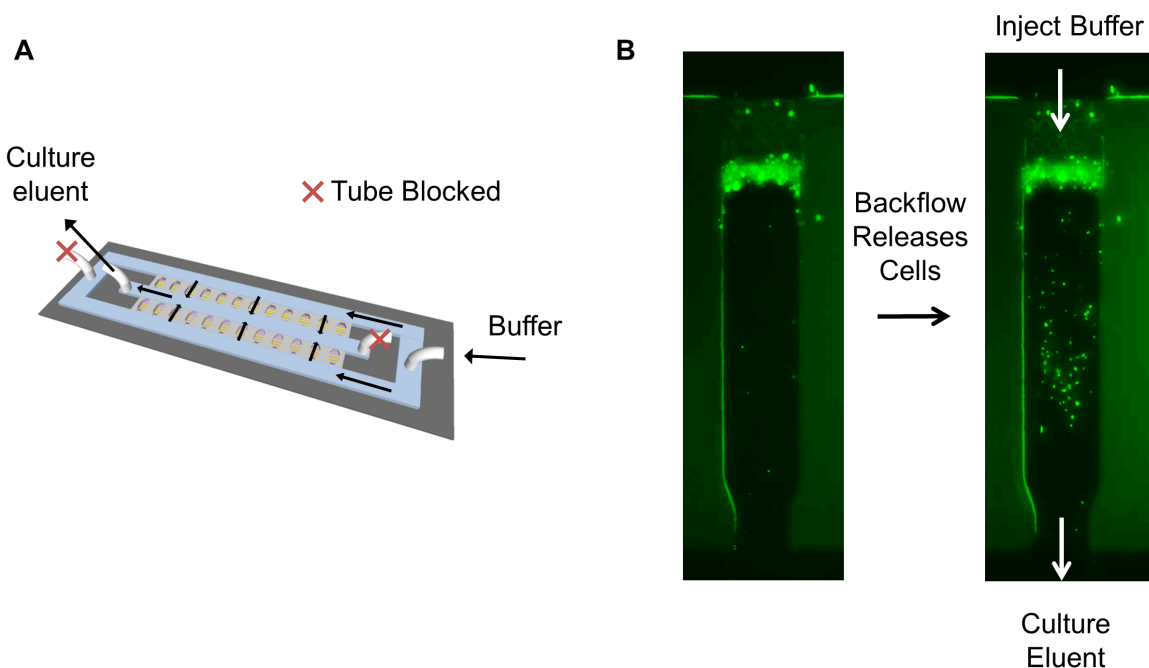


Figure S9. Eluting bacteria for off-chip culture. (A) Bacteria can be eluted by flowing buffer from the outlet towards the inlet and directing the flow backwards through the filters. (B) Image of *E. coli* expressing green fluorescent protein (GFP) captured in the in-well filter before and after applying a backflow.

Effect of electrodeposition and surface fouling on the on-chip electrodes

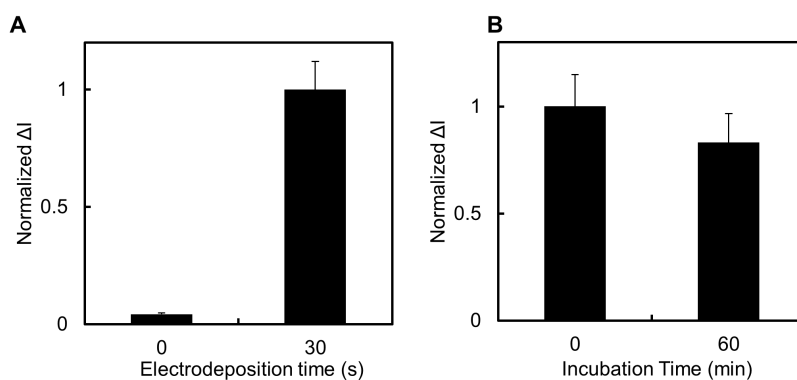


Figure S10. Effect of electrodeposition and surface fouling on the on-chip electrodes. (A) Electrochemical scans on-chip of 1 mM resazurin before and after electrodepositing Au for 30 s. Electrodeposition increases the electrode surface area and thus the magnitude of the current. (B) Effect of surface fouling on the electrodes. After incubating for one hour, we observe a slight signal decrease when scanning on-chip with 1 mM resazurin. Currents are normalized to the maximum current.

Time required for antibiotics to inhibit bacterial metabolic activity

In order to choose a suitable incubation period for the susceptibility test, we studied the time required for the antibiotics to begin inhibiting bacterial metabolic activity. To study this, we used a high concentration of bacteria in order to determine the minimum time required for the bacteria to exhibit differential metabolic activity in response to the tested antibiotics. *K. pneumoniae* at 1×10^5 cfu/ μ L were incubated at 37°C in the presence of ampicillin and ciprofloxacin at 100 μ g/mL in LB media and 1 mM resazurin. The increase in fluorescence induced by the conversion of resazurin by metabolically active bacteria was recorded. We find that the signal from the sample incubated with ciprofloxacin is suppressed within 30 minutes indicating that the antibiotic rapidly inhibits the metabolism of *K. pneumoniae*. As this strain of *K. pneumoniae* is resistant to ampicillin, the fluorescence increases as *K. pneumoniae* convert resazurin. These results indicate that the chosen incubation period of 60 minutes for the rapid on-chip susceptibility test is sufficiently long for the bacteria to exhibit differential metabolic activity in response to the tested antibiotics.

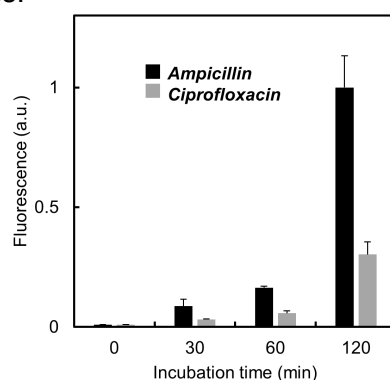


Figure S11. Time required for antibiotics to hinder the metabolic activity of bacteria. *K. pneumoniae* at 1×10^5 cfu/ μ L were incubated with 1 mM resazurin and ampicillin and ciprofloxacin at 100 μ g/mL. When incubated with ciprofloxacin, the signal is suppressed compared to that from ampicillin, indicating that ciprofloxacin reduces the metabolic activity of *K. pneumoniae*. This difference in metabolic activity is detectable within 30 minutes indicating the antibiotics rapidly inhibit the metabolism of the bacteria.

Correlation between the microdilution susceptibility test and the on-chip assay

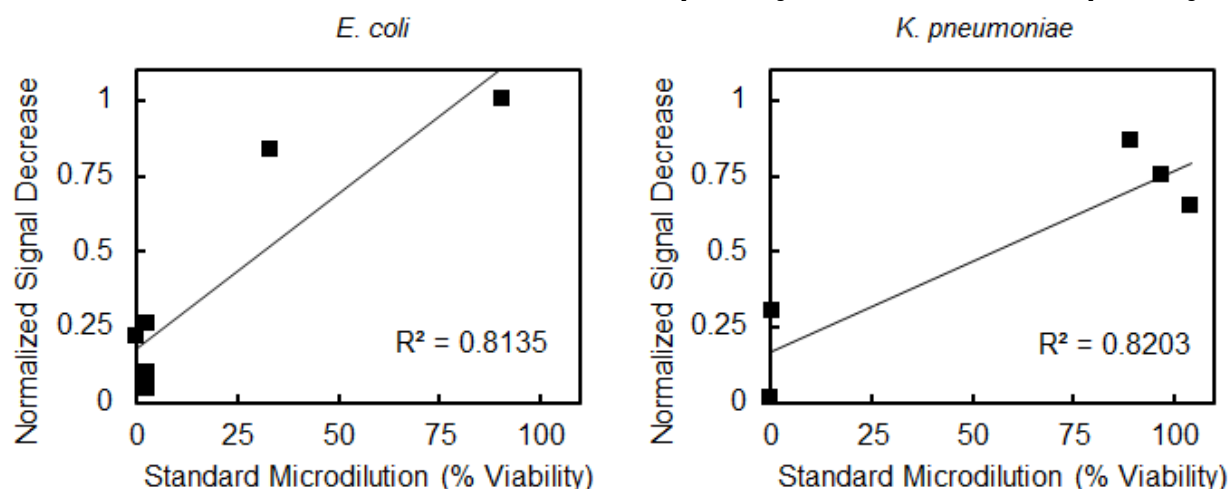


Figure S12. Correlation between the standard microdilution susceptibility tests and the on-chip assay. We found good correlation between our measurements and standard assays with r^2 values of 0.81 and 0.82 for *E. coli* and *K. pneumoniae* respectively. Outliers were removed using a Modified Thompson Tau test.

Optimization of in-line urine sample processing

To test undiluted urine on chip, we devised a method to remove large particulates from urine while allowing bacteria to pass through the filter. We tested various pre-filter sizes to ensure that bacteria spiked in whole urine could be recovered. We spiked *E. coli* at 1×10^2 cfu/ μ L into whole urine and passed 100 μ L of the urine through the pre-filters with various pore diameters. We plated the filtrate on agar plates and incubated the plates overnight at 37°C. We counted the number of bacterial colonies and found that using a 10 μ m pre-filter, nearly 75% of bacteria could be recovered directly from whole urine.

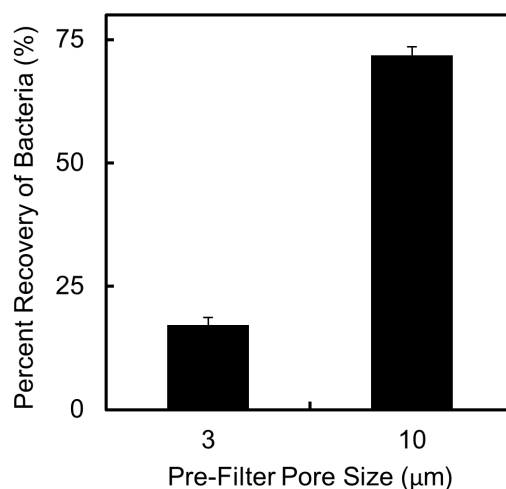


Figure S13. Effect of pre-filter size on recovery of bacteria spiked in undiluted urine. *E. coli* can be efficiently recovered directly from urine using a pre-filter with a 10 μ m pore diameter.

On-chip electrochemical scans from susceptibility testing

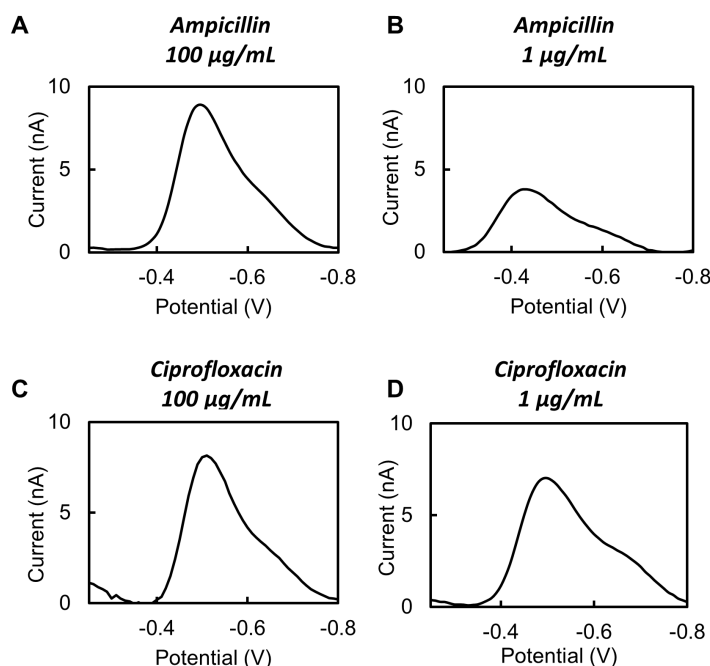


Fig. S14. On-chip electrochemical scans acquired from *E. coli* spiked in complex matrices. Electrochemical scans acquired on chip after introducing *E. coli* spiked in urine at 100 CFU/ μ L and subsequently introducing 1 mM resazurin and an antibiotic. Samples were incubated for 60 min at 37°C. *E. coli* were incubated with (A) 100 μ g/mL ampicillin, (B) 1 μ g/mL ampicillin, (C) 100 μ g/mL ciprofloxacin, and (D) 1 μ g/mL ciprofloxacin. In the presence of 1 μ g/mL ampicillin, the magnitude of the signal is reduced as that concentration of ampicillin is insufficient to inhibit the metabolism of *E. coli*. Electrochemical scans were acquired on-chip with the on-chip Au reference electrode. Peaks are shifted to more negative potentials when compared to using the Ag/AgCl reference electrode.

References

1. R. Greenwood, P. F. Luckham, and T. Gregory, *J. Colloid Interface Sci.*, 1997, **191**, 11–21.