Electronic Supplementary Material (ESI) for Lab on a Chip.


# Multiparameter antibiotic resistance detection based on hydrodynamic trapping of individual *E. coli*.

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## Supplementary information

### Plasmid selection and bacteria transformation

The following plasmids were used for generation of antibiotic resistant *E. coli* MG1655: a pBAD vector containing *ampR* for ampicillin resistance, a pET vector containing *kanR* for kanamycin resistance; and pT2ST containing *ampR* and *dfrA* for ampicillin and trimethoprim resistance respectively. pT2ST was a gift from Shelley Copley[1] (Addgene plasmid # 59384; http://n2t.net/addgene:59384 ; RRID:Addgene_59384).

For the transformation, *E. coli* MG1655 was grown overnight in LB at 37°C. Cells were made electro competent by growing to an OD ≈ 0.6 then washing 3 times in ice-cold 10% glycerol before re-suspending in 100µl of 10% glycerol. Electroporation was performed with a Bio-Rad Multipulse Electroporator using 50µl of prepared cells and 40-150ng of plasmid. Shocked cells were added to 1ml of LB broth warmed to 37°C and then incubated at 37°C for 2 hours. 10µl of culture was then plated onto either 100µg/ml ampicillin or 60 µg/ml kanamycin plates and incubated for 24 hours. Single colonies were picked from these plates for creation of glycerol stocks and were used in all subsequent experiments.

### Growth curve assay

Wild type *E. coli* MG1655 and the three transformed strains are grown overnight in LB with appropriate antibiotics for plasmid selection. Cells are washed three times in both growth medias then re-suspended in fresh LB or MH before being diluted to 0.1 OD to give standardised suspension of $10^8$ cfu/ml. The standardised suspension was diluted 1:100 then 100µl added to each well to give a final bacteria concentration of 5.5 x$10^5$ cfu/ml. Growth curve assays were performed using a Biotek Synergy plate reader at 37°C over 24 hours, with orbital shaking at 200 RPM and an OD reading at 650 nm taken every 20 minutes. Results shown in figure S4 are an average of 5 replicates, normalised to blank media controls.

### Microdilution resazurin assay

The resazurin-based microdilution assay is employed as a method to detect bacteria growth in the presence of antibiotics and to determine the MIC of different drugs[2,3]. Ampicillin, kanamycin and trimethoprim were diluted from stock solutions into MHB at 1024 µg/ml. Column 1 of a standard 96-well plate was used as a no antibiotic control, columns 2-11 increased in antibiotic concentration from 1 - 512 µg/ml and column 12 contained only MHB as a sterility control (see an example in figure S2(a)). For the antibiotic serial dilution, 50µl of the 1024 µg/ml antibiotics was first added to the 512ug/ml column, mixed well using a multichannel pipette before transferring to the next well and repeated to give a final concentration of 1µg/ml in column 2.

Wild type *E. coli* MG1655 and the three transformed strains are grown overnight in LB with appropriate antibiotics for plasmid selection. Cells were washed three times in MH then re-suspended in MH before first being diluted to 0.1 OD to give standardised suspension of $10^8$ cfu/ml. The standardised suspension was diluted 1:100 then 50µl added to each well to give a final bacteria concentration of 5.5 x$10^5$ cfu/ml. 50µl MHB was added to column 12 to give 100µl of solution in all wells. After overnight incubation at 37°C, 30µl of 0.015% solution of resazurin in MHB is introduced in each well and incubated for 4 hours. Results are validated by visual inspection of the wells as well as by measuring the optical absorbance of each well at 605nm. A picture of the wells and values of absorbance are reported in figure S2 (for the trimethoprim and

ampicillin resistant *E. coli*) and S3 (for a comparison between the wild type *E. coli* MG1655 and the three transformed strains). High levels of absorbance at 605nm imply that the resazurin has not been reduced to resorufin, meaning that bacteria are not viable, and the corresponding inoculum appears dark blue/purple. Conversely, if bacteria are still viable, most of the resazurin has been reduced to resorufin, resulting in low absorbance at 605nm and in a pink solution.

## Description of supplementary videos

**Movie S1**. Method for retrieving the pixel intensity traces from the videos of the experiment. The left-hand side shows a trapping event of a motile *E. coli* MG1655. The average pixel intensity is evaluated over the blue dashed box and plotted over time in the graph on the right-hand side. The increase in the standard deviation of the signal due to the movement of the bacterium within the detection area is clear. The video is played at 2x speed.

**Movie S2**. A short and round *E. coli* MG1655 manages to rotate inside the trap and gain access to the vertical gap, thus escaping. The video is played at 0.5x speed to emphasize the rotation inside the trap.

**Movie S3**. An elongated *E. coli* MG1655 is not able to rotate inside the trap, thus it remains trapped for an extended period of time. The video is played at normal speed.

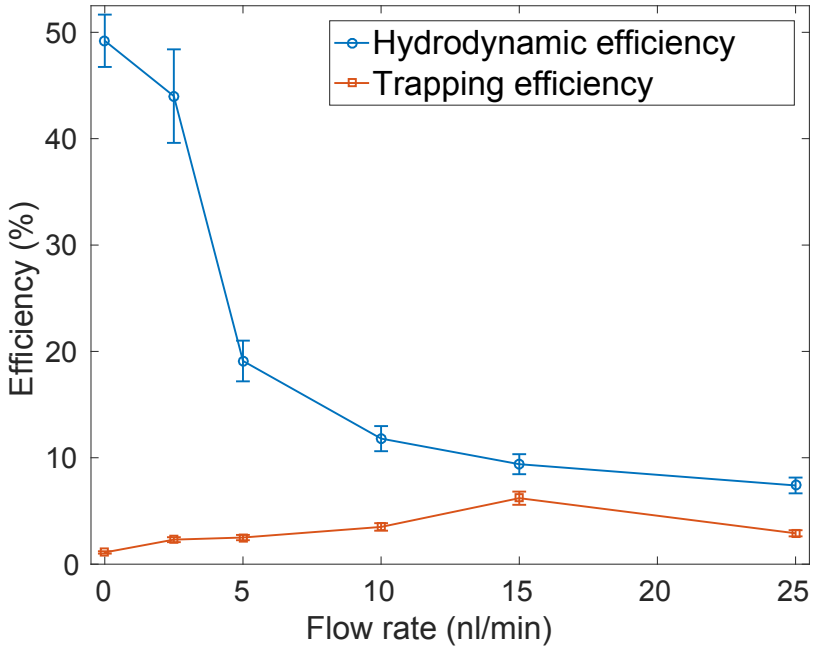


**Figure S1.** Hydrodynamic (blue curve with circle markers) and trapping efficiency (orange curve with square markers) as a function of the flow rate for the motile *E. coli* MG1655 strain. The hydrodynamic efficiency quantifies the fraction of bacteria entering a trap, regardless of the amount of time spent inside. Conversely, the trapping efficiency accounts for bacteria remaining trapped for at least 1 minute. At low flow rates, and in the limiting case of no flow, approximately half the bacteria enter a trap since they are able to explore the environment more efficiently and swim almost unbiasedly. In this scenario, however, cells do not stay trapped for a long time as they can easily swim against the flow (hence the low values of trapping efficiency). As the flow rate increases, fewer swim into a trap because of a net velocity being imparted by the fluid and party because of the shear trapping phenomenon. However, those that enter a trap are more likely to stay trapped, as shown by the growing trapping efficiency. At around 15 nl/min the two efficiencies become as close as allowed by the two competing effects, reaching a value just below 10%. At higher flow rates both figures decrease as bacteria are preferentially dragged by the fluid and are not able to efficiently migrate across streamlines and swim into a trap.

## Supplementary figures

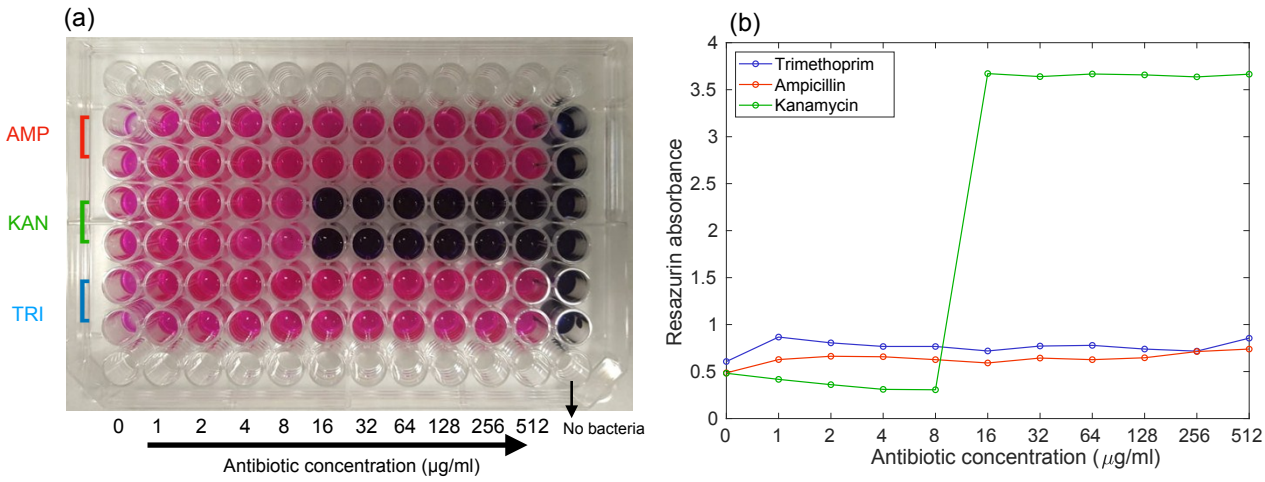


**Figure S2.** **(a)** Example of a 96-well plate resazurin microdilution assay conducted on the trimethoprim and ampicillin resistant *E. coli* MG 1655. The concentration of the three antibiotics is increased along the columns, whereas different rows correspond to different antibiotics. Each antibiotic has been tested in duplicate, as indicated by the labels on the left-hand side. The last column is used as a control with no bacteria in the wells. The top and bottom pair of rows, corresponding to ampicillin and trimethoprim, show pink wells for every concentration of antibiotics. This means that bacteria are resistant and still viable after exposure to the antibiotic, since they have been able to fully reduce the resazurin to resorufin. The middle rows show a breakpoint at 16 µg/ml, corresponding to the MIC range for kanamycin of this particular strain. **(b)** reports the relative values of absorbance measured at 605nm. A low level of absorbance corresponds to a pink well (i.e. to viable bacteria) because of the resorufin absorbance peak being blue-shifted to 573nm. This is the case for TRI and AMP (blue and red curves) at every antibiotic concentration. High absorbance is obtained for blue wells and is indicative of inhibited growth, as obtained for KAN above 8µg/ml.

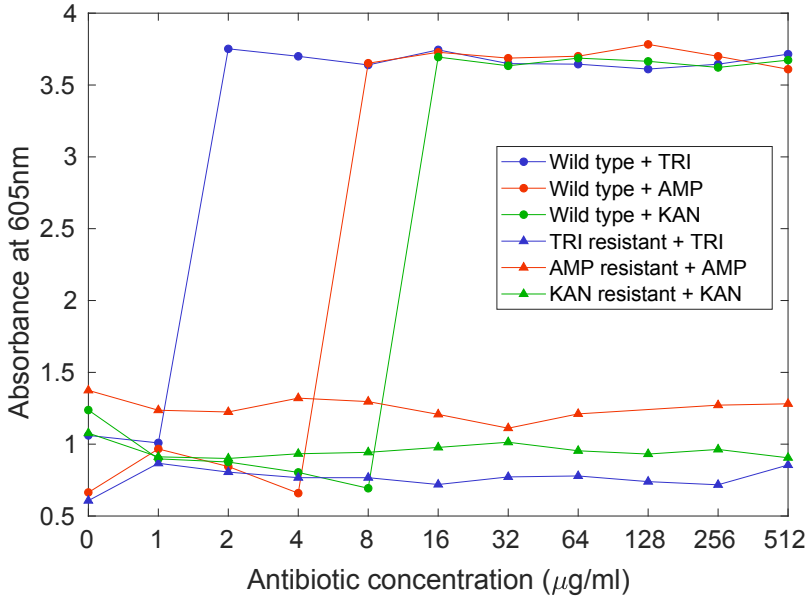


**Figure S3.** Optical absorbance at 605nm after overnight incubation with trimethoprim (TRI, blue curves), ampicillin (AMP, red curves) and kanamycin (KAN, green curves) administered to wild type *E. coli* MG 1655 (circles) and transformed *E. coli* MG1655 to confer resistance (triangles). Successful acquisition of resistance is confirmed by the low values of absorbance at all concentrations for the transformed strains grown in the presence of the corresponding antibiotics (triangles). The curves for the wild type *E. coli* (untransformed strain, circles) are used to determine the minimum inhibitory concentration (MIC) values for the different antibiotics, which correspond to the breakpoints observed in the curves. In particular the MIC for TRI is in the range 1-2µg/ml, for AMP is 4-8 µg/ml, while for KAN is 8-16 µg/ml.

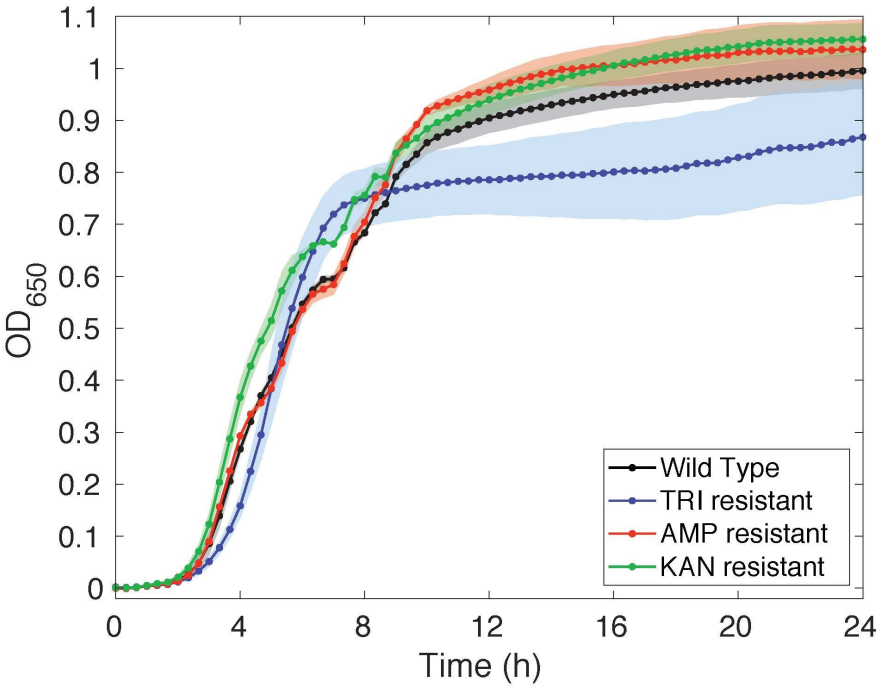


**Figure S4.** Growth curves for the wild type *E. coli* MG1655 (black curve) and transformed *E. coli* MG1655 resistant to trimethoprim (TRI, blue curve), ampicillin (AMP, red curve) and kanamycin (KAN, green curve). Growth is followed by measuring the optical density at 650nm ($OD_{650}$). Each curve is the average of 5 replicates with the error bars representing the standard error between the replicates. The curves confirm that the transformation process that made the strain resistant to different antibiotics has not significant effects on bacteria growth.

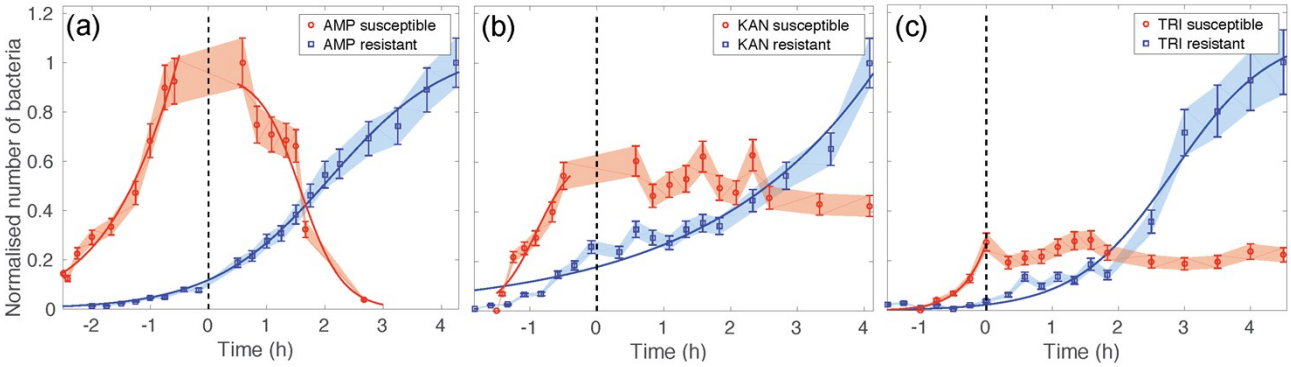


**Figure S5.** Normalised number of bacteria swimming through the microfluidic channel over time for susceptible (red curves with circle markers) and resistant (blue curves with square markers) *E. coli* MG 1655 in the presence of 10 µg/ml ampicillin (panel a), kanamycin (panel b) and trimethoprim (panel c). Negative times serve as no-drug controls as antibiotics are administered at time t=0. The graphs clearly show that the growth of the susceptible strains is affected by the antibiotics (red curves). In particular, ampicillin is causing the number to drastically decrease, given its bactericidal mode of action which leads to cell lysis. Instead, trimethoprim is bacteriostatic, as shown by the division being inhibited and the number of bacteria remaining constant over time after the introduction of the drug. The same effect is observed for kanamycin, which is bactericidal and prevents bacteria division too. On the other hand, the growth of resistant strains is not affected by the antibiotics and it follows a logistic growth curve, illustrated by the fitted blue continuous lines and the blue square data points.

## Supplementary references

1 J. Kim, A. M. Webb, J. P. Kershner, S. Blaskowski and S. D. Copley, *BMC Biotechnol.*, 2014, **14**, 1–13.

2 S. D. Sarker, L. Nahar and Y. Kumarasamy, *Methods*, 2007, **42**, 321–324.

3 A. J. Drummond and R. D. Waigh, *Recent Res. Dev. Phytochem.*, 2000, **4**, 143–152.