Electronic Supplementary Material (ESI) for Analyst.

Supporting Information:

Miniaturised broth microdilution for simplified antibiotic susceptibility testing of Gram negative clinical isolates using microcapillary devices

An array of up to 12 test strips were clipped into a ‘ladder’ holder with a 9mm pitch. Each test strip contains 10 capillaries each loaded to release doubling dilutions of antibiotic. Test strip arrays were dipped directly into 96-well microtitre plate wells allowing 1 microlitre of the sample to be drawn up by capillary action into all 10 microcapillaries. Plastic end covers filled with silicone grease were slid over the ends of the test strips, sealing the capillary ends to prevent evaporation. Each well of a 96 wellplate contains a different isolate that is expanded into a 10-plex AST using the microcapillary test. Each ladder of microcapillary BMD test strips provides up to 16X higher throughput than microtitre plates. Bacteria growth and antibiotic inhibition is determined by resazurin color change. Blue to pink/white indicates growth and if antibiotic is present, indicates that concentration is below the minimum inhibitory concentration (MIC). At or above MIC, the capillary remains blue.

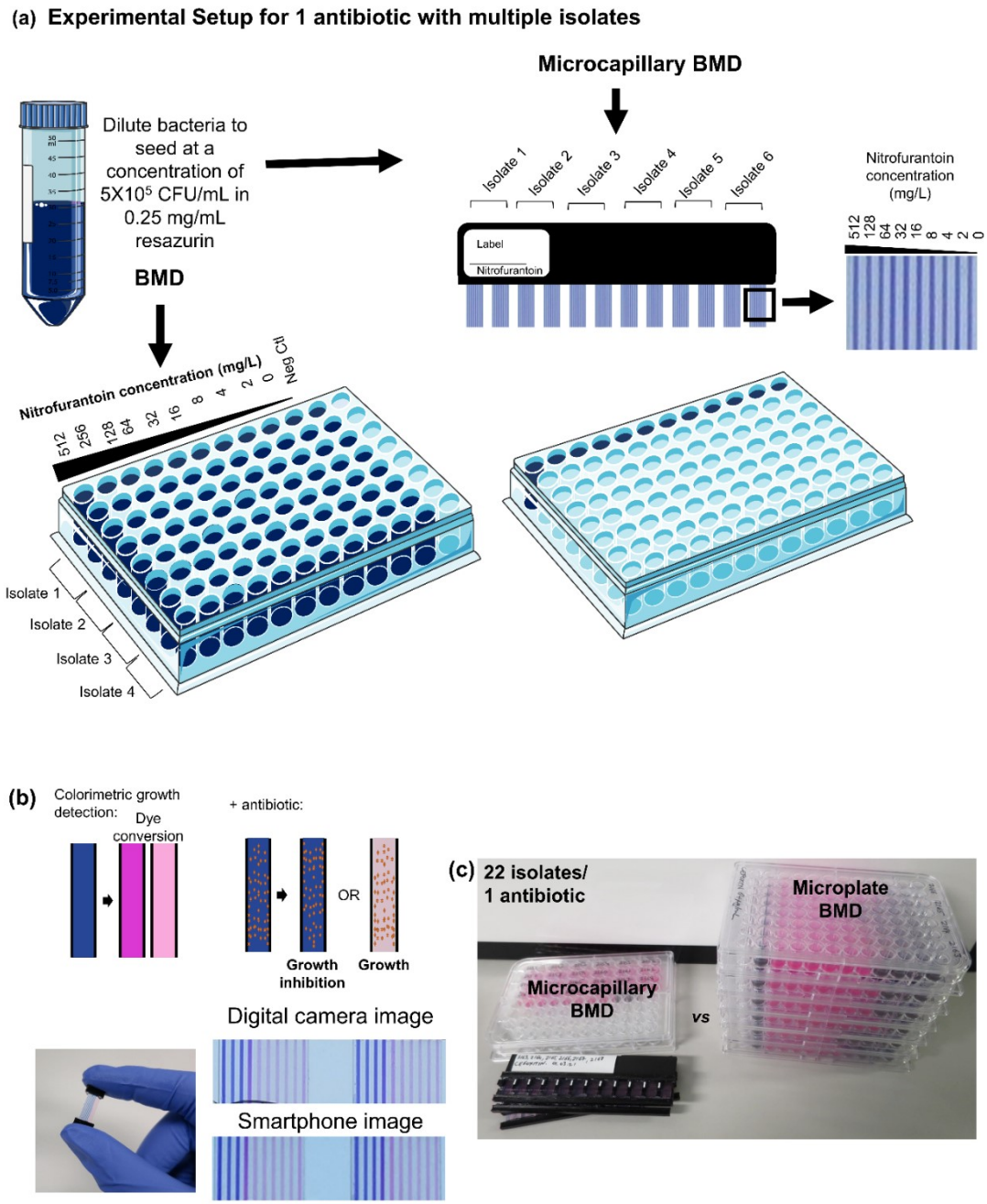
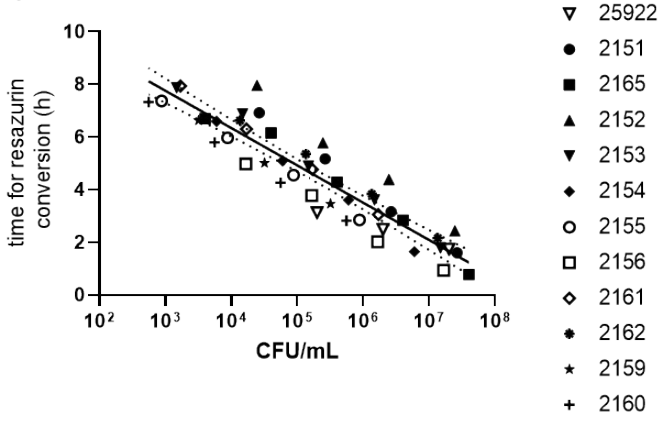


Figure S1. Microcapillary test use. (a) Microcapillary test use compared to standard microplate BMD. Image created using servier medical art **(b)** Bacteria growth and antibiotic inhibition is determined by resazurin color change. Blue to pink/white indicates bacterial growth **(c)** Image illustrates the number of plates vs microcapillaries required to test 22 isolates in duplicate for a single antibiotic at 9 concentrations plus no antibiotic.

(a)



(b)

	Time (h)		
	Mean	Upper 95% CI	Lower 95% CI
$2X10^5$ CFU/mL	4.5	4.7	4.3
$8X10^5$ CFU/mL	3.6	3.9	3.3

Figure S2. Time to resazurin conversion is plotted against CFU/ml determined by overnight spot dot. Shaded area indicates CLSI standard inoculum range for MTP BMD. Solid line indicates linear regression and dotted lines indicate 95% confidence intervals **(e)** Table indicates the average time to growth detection at two different inoculum cell densities, and 95% confidence intervals for detection time. Data points indicate the average of 3-10 capillaries

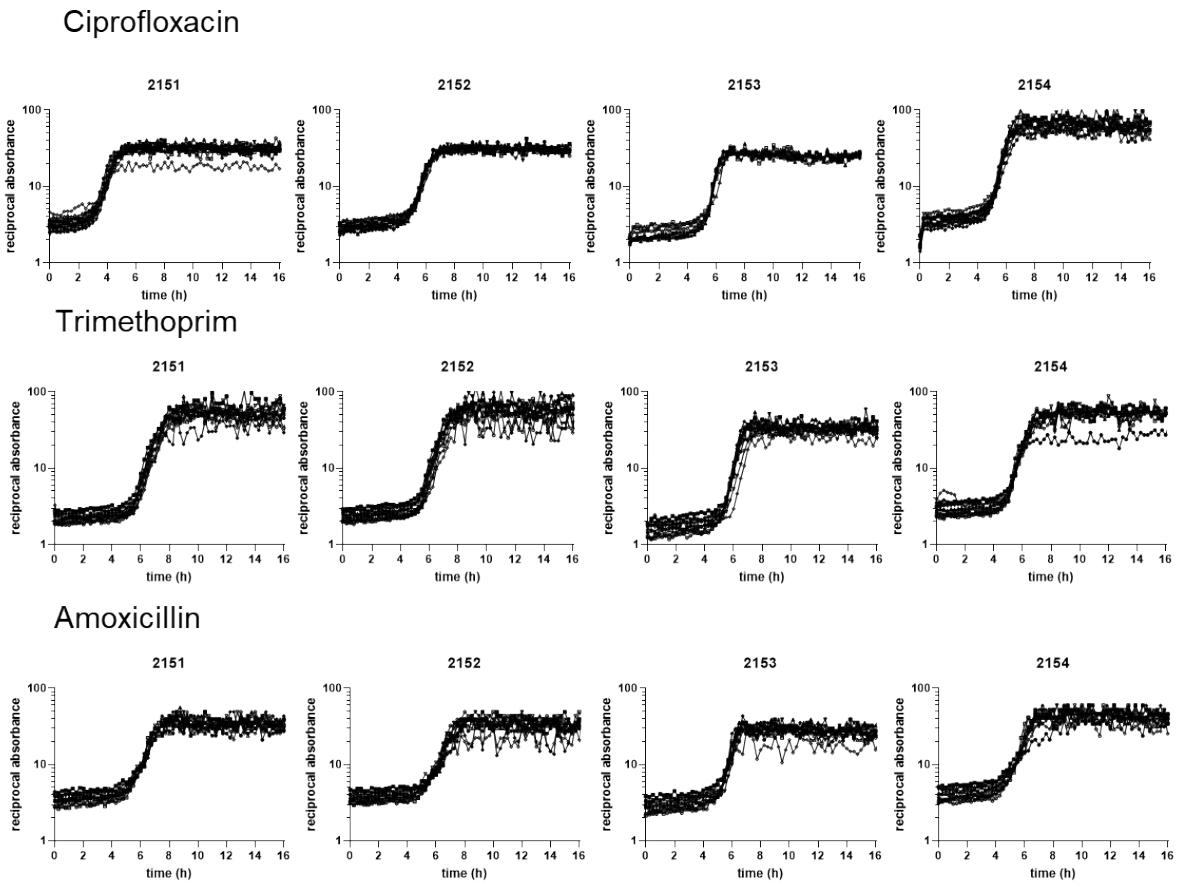


Figure S3. Representative growth curves of ciprofloxacin, trimethoprim and amoxicillin resistant isolates. Data indicates full MIC growth kinetics. Antibiotics start at 1 mg/L, 32 mg/L and 64 mg/L for ciprofloxacin, trimethoprim and amoxicillin respectively and have a series of nine, 2-fold dilutions and a no antibiotic control. For resistant isolates no delayed growth was observed in the antibiotic contained capillaries compared to the no antibiotic control.

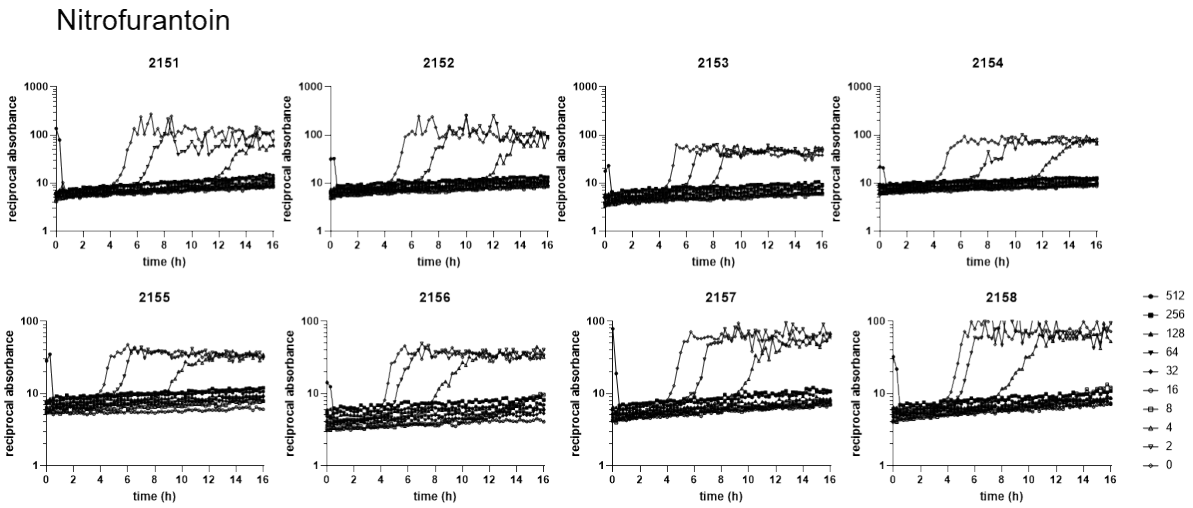


Figure S4. Representative growth curves of nitrofurantoin susceptible isolates. Data indicates full MIC growth kinetics. Antibiotics start at 512 mg/L and have a series of nine, 2-fold dilutions and a no antibiotic control. The first line of growth observed is the no antibiotic growth control. Significant delay was observed between the no antibiotic control and antibiotic containing capillaries below the MIC.

Table S1. EUCAST v 12.0 antibiotic MIC breakpoints used for categorical analysis

Number	Antibiotic	EUCAST MIC breakpoints (mg/L) ≤S/>R	ATU
1	Cefoxitin	≤8	-
2	Ciprofloxacin	≤0.25	0.5
3	Trimethoprim	≤4	-
4	Nitrofurantoin	≤64	-
5	Cephalexin	≤16	-
6	Amoxicillin	≤8	-
7	Amikacin	≤8	-
8	Gentamicin	≤2	-
9	Fosfomycin	≤8	-
10	Cefuroxime	≤8	-
11	Amoxicillin-clavulanic acid	≤32	-
12	Ofloxacin	≤0.25/>0.5	-
13	Ceftazidime	≤1/>4	-
14	Co-trimoxazole	≤2/>4	-
15	Meropenem	≤2/>8	-
16	Cefotaxime	≤1/>2	-
17	Ertapenem	≤0.5	-

Effect of resazurin on broth microdilution

Bacterial growth was monitored using the metabolic indicator resazurin. Resazurin has been used in multiple plate assays studying bacterial and cellular growth, mitochondrial function, and MIC of antimicrobial agents. However, with many cell viability test methods the resazurin is added towards the end of the experiment and incubated for several hours to measure metabolic activity after a primary incubation period with antimicrobial agent but no dye, to reduce the risk of the dye interacting with the antimicrobial (Kim and Jang, 2018, Teh et al., 2017, Rakhmawatie et al., 2019, Elshikh et al., 2016, Sarker et al., 2007). In contrast, for the novel microcapillary BMD method, the resazurin is present from the start. Furthermore, the capillaries in the MCF test strips have a 20-fold shorter light pathlength than a microtitre plate, therefore the concentration of resazurin had to be high enough to be clearly visible in the capillaries. Microplate BMD with the addition of resazurin at the start was therefore compared to microplate BMD without dye to evaluate if the presence of resazurin dye affected antimicrobial susceptibility and MIC results. There was 100% essential agreement (MIC within ± $\log_2$ dilution of antibiotic) when microplate BMD was compared with and without resazurin dye for the MIC values of nitrofurantoin and cephalexin (S1 Dataset). The Vitek 2 system to which the microcapillary BMD was also compared uses turbidimetry rather than dyes to detect growth. The agreement between these methods indicate resazurin presence does not interfere with MIC determination for *E. coli* and *K. pneumoniae* when mixed with the sample at 0.25 mg/mL and incubated overnight. Although small, the individual capillaries are large enough to be scored by the naked eye if digital camera is not available to record result.

Table S2. Microplate BMD compared to microplate BMD and Microcapillary BMD in the presence of resazurin

		MIC (µg/mL)					
		Nitrofurantoin			Cephalexin		
		Microplate BMD	Microplate BMD + resazurin	Microcapillary BMD + resazurin	Microplate BMD	Microplate BMD + resazurin	Microcapillary BMD + resazurin
Ref Strain	ATCC 25922	4	4	8	16	16	16
	NCTC 13352	<2	<2	8	16	16	8
UPEC Isolate ID	2151	8	8	8	>64	>64	>64
	2152	8	4	8	>64	>64	>64
	2153	8	8	8	32	64	32
	2154	8	8	8	>64	>64	>64
	2155	8	8	8	32	32	64
	2156	16	16	16	>64	>64	>64
	2157	16	8	8	64	64	64
	2158	16	16	8	>64	>64	>64
	2159	16	16	16	>64	>64	>64
	2160	8	8	8	>64	>64	>64
	2161	8	4	8	>64	>64	>64
	2162	16	8	16	>64	>64	>64
	2163	4	4	4	>64	>64	>64
	2164	8	8	16	>64	>64	>64
	2165	4	≤2	4	8	8	8
	2166	16	16	16	>64	>64	>64
	2167	8	4	8	>64	>64	>64
	2168	8	4	8	>64	>64	>64
	2169	4	≤2	4	>64	>64	>64
	2170	128	64	64	>64	>64	>64

Table S3. AST agreement between overnight microplate BMD and 6 h microcapillary BMD for *E. coli* and *K. pneumoniae* isolates from urine.

Antibiotic	Prevalence of resistance (%)	Categorical Agreement (%)	Minor Errors (%)	Major Errors (%)	Very Major Errors (%)	Growth not detected*
Trimethoprim	85% (23/27)	96% (26/27)	0	0	0	4% (1/27)
Cephalexin	86% (19/27)	100% (27/27)	0	0	0	-
Amoxicillin	95% (26/27)	100% (27/27)	0	0	0	-
Nitrofurantoin	4% (1/27)	96% (26/27)	0	0	4% (1/27)	-
Ciprofloxacin	81% (22/27)	93% (25/27)	0	0	7% (2/27)	-
Amikacin	30% (8/27)	81% (22/27)	0	0	19% (5/27)	-
Cefoxitin	66% (18/27)	70% (19/27)	0	0	26% (7/27)	4% (1/27)
Gentamicin	74% (20/27)	56% (15/27)	0	0	44% (12/27)	-
Fosfomycin	4% (1/24)	96% (26/27)	0	0	4% (1/27)	-
Cefuroxime	89% (24/27)	89% (24/27)	0	0	11% (3/27)	-
Amox-clav	78% (21/27)	74% (20/27)	0	0	26% (7/27)	-
Ofloxacin	89% (24/27)	70% (19/27)	19% (5/27)	0	7% (2/27)	4% (1/27)
Cefotaxime	88% (21/27)	96% (26/27)	0	0	0	4% (1/27)
Ceftazidime	88% (21/27)	92% (25/27)	4% (1/27)	0	0	4% (1/27)
Co-trimoxazole	84% (21/25)	96% (26/27)	0	0	0	4% (1/27)
Meropenem	7% (2/27)	96% (26/27)	4% (1/27)	0	0	-
Ertapenem	11% (3/27)	100% (27/27)	0	0	0	-

*All occurred with *E. coli* 13352

Table S4. Determination of endpoint MIC using varying inoculum density.

UPEC 2165						
	Nitrofurantoin		Cefalexin		Ciprofloxacin	
CFU/mL	Endpoint Microplate BMD ($5X10^5$CFU/mL)	Microcapillary BMD	Endpoint Microplate BMD ($5X10^5$CFU/mL)	Microcapillary BMD	Endpoint Microplate BMD ($5X10^5$CFU/mL)	Microcapillary BMD
$4X10^7$		>512 (R)		> 64 (R)		>1 (R)
$4X10^6$		8 (S)		16 (S)		1 (R)
$4X10^5$	4 (S)	4 (S)	8 (S)	16 (S)	0.25 (S)	0.25 (S)
$4X10^4$		≤2 (S)		16 (S)		0.25 (S)
$4X10^3$		≤2 (S)		16 (S)		0.25 (S)
E. coli 25922						
$2X10^7$		64 (S)		>64 (R)		1 (R)
$2X10^6$		4 (S)		>64 (R)		0.06 (S)
$2X10^5$	4 (S)	4 (S)	16 (S)	32 (R)	0.015 (S)	0.015 (S)
$2X10^4$		4 (S)		16 (S)		0.03 (S)
$2X10^3$		4 (S)		16 (S)		0.015 (S)