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

Supplementary Information

Fast Antimicrobial Susceptibility Testing on *Escherichia coli* by Metabolic Heat Nanocalorimetry

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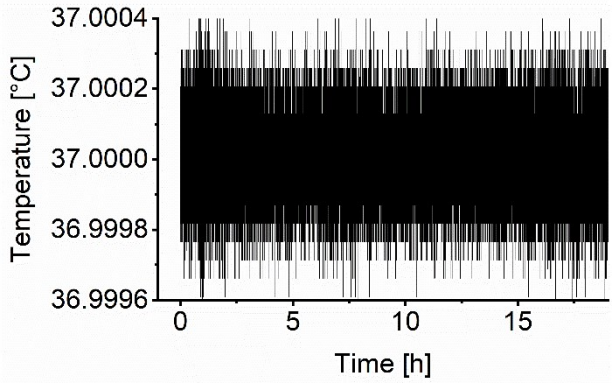

Fig. S1. Temperature stabilization of the INCfAST platform. The plot indicates that the temperature fluctuation is about ±250 µK around the set point of 37 °C for the duration of a typical metabolic assay. The temperature was measured with the RTD sensor of the platform, which is located close to the microincubator.

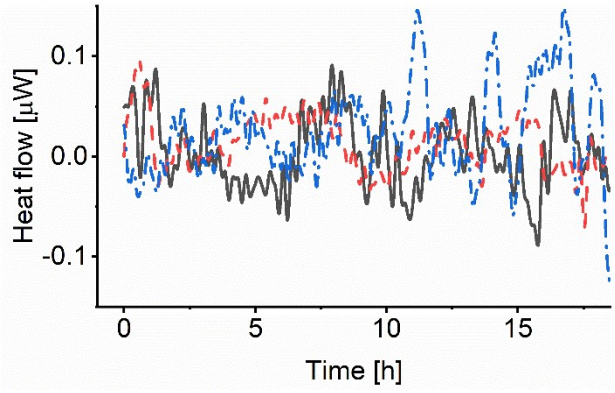

Fig. S2. Control experiments without bacteria. In order to evaluate the efficiency of the cleaning and sterilization protocol applied to the fluidic system of the platform, control curves have been recorded with pure MH (no bacteria, $n = 3$). Heat flow signals fluctuate around zero-level (after baseline correction) for the time scale of typical assays, indicating that the

microincubator is well sterilized and that the recorded metabolic heat signals are not generated by any contamination with other bacteria. An average filter was firstly applied to process these curves. From these recordings we derive a maximum noise level of 200 - 250 nW, resulting in a conservative limit of detection (LOD) estimate of ~750 nW for metabolic heat measurements (corresponding to SNR ≈ 3).

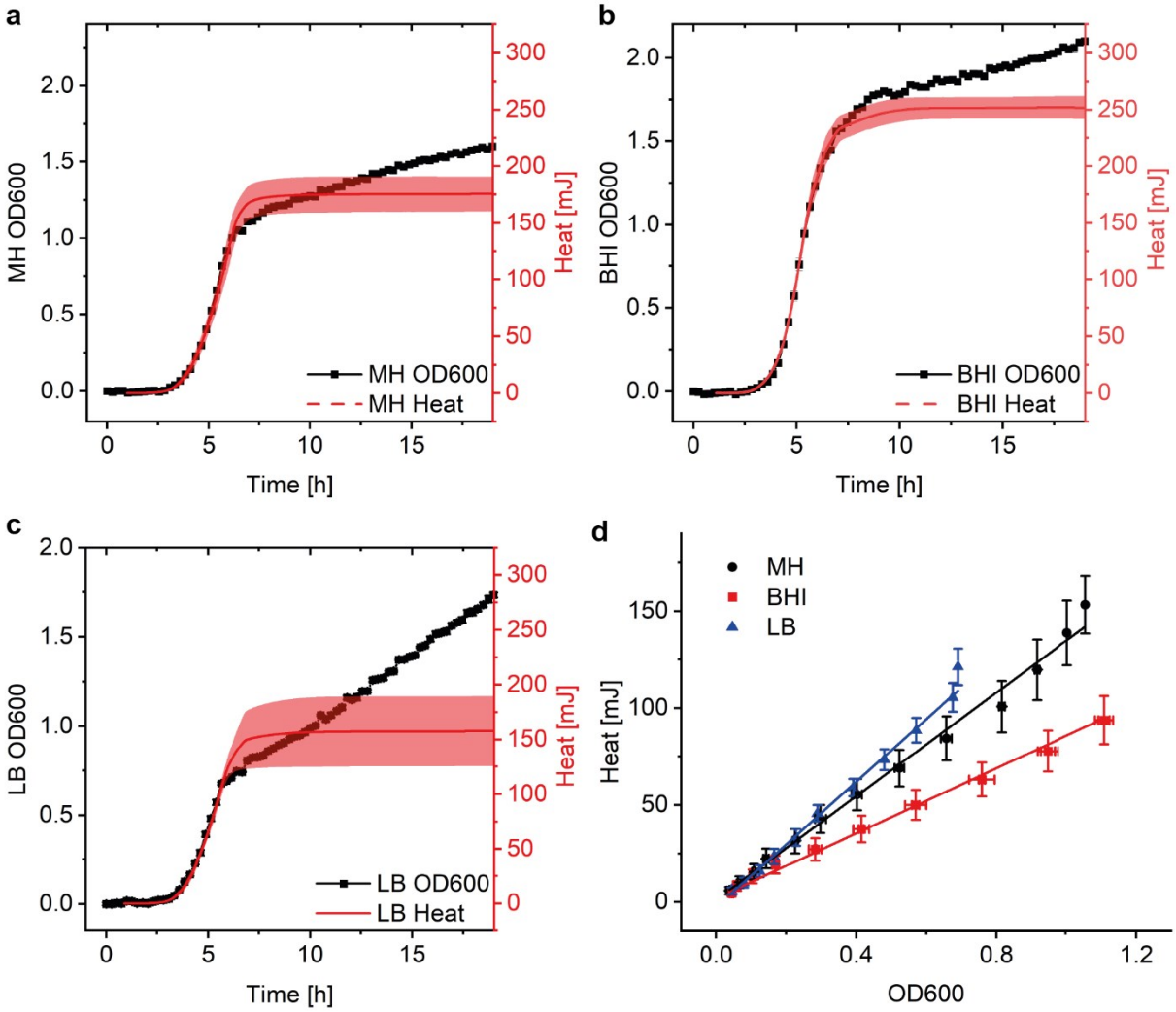

Fig. S3. Comparison of metabolic heat curves with optical density measurements in different culture media (*E. coli* ATCC 25922). (a) MH, (b) BHI, and (c) LB at 37 °C, respectively. OD600 curves have been recorded using a plate reader (PerkinElmer VICTOR 3®). Heat signal and OD600 curves overlap well in the exponential growth regions (*i.e.* up to $t \approx 5$ h - 6 h), after time synchronization and scaling with the heat/OD600 coefficients derived from (d).

Time synchronization is required as initial delay times t_{delay} depend on the exact culture conditions and thus are different for OD600 and heat curves. Time synchronization was carried out by manually adjusting the midpoint of the exponential growth region, *i.e.* by shifting the heat curve in order to overlap with the OD600 curve. (d) Bacterial growth in different culture media affects the metabolic heat production. In the exponential growth phase, a specific heat/OD600 coefficient could be determined for each medium by linear regression of the heat signal vs OD600 data points (134 mJ/1.0 OD600 for MH, 84 mJ/1.0 OD600 for BHI and 162 mJ/1.0 OD600 for LB, respectively). This proportionality indicates that the heat curves recorded with the INCfAST platform can be understood as growth curves after calibration with respect to biomass. In contrast to heat curves, OD600 curves continue increasing in a roughly linear way above the exponential growth phase in all cases (Fig. S3-S5), however this region is not relevant for the present analysis. The continuous OD600 increase may be attributed to ongoing oxygen replenishment due to shaking of the culture plates on the plate reader, whereas oxygen is depleted in the microincubator. All curves represent mean ± SE (n = 3).

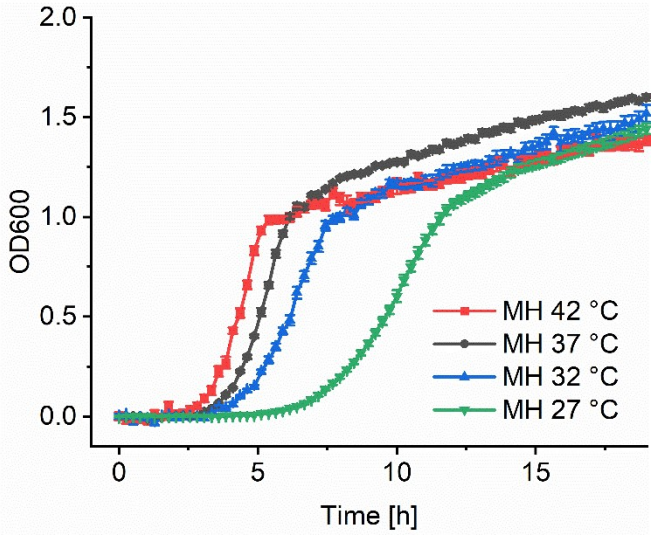

Fig. S4. Bacterial growth curves based on optical density measurements for culture at different temperatures in MH (*E. coli ATCC 25922*). Lag phase elongation for decreasing

culture temperatures, in particular for 27 °C, can be observed in accordance to the heat curves shown in Fig. 3e. The biomass after overnight culture approaches comparable levels for all conditions. OD600 measurements have been carried out on a plate reader (PerkinElmer VICTOR 3®). All curves represent mean ± SE (n = 3).

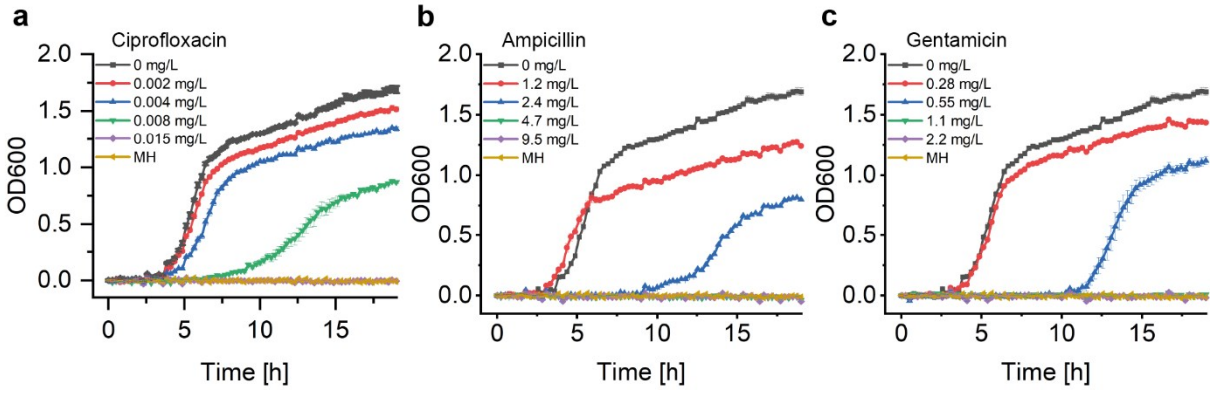

Fig. S5. Growth curves based on optical density measurements for bacterial cultures with different antimicrobial conditions in MH (*E. coli ATCC 25922*). OD600 measurements have been carried out on a plate reader (PerkinElmer VICTOR 3®) during bacterial growth in the presence of (a) ciprofloxacin, (b) ampicillin and (c) gentamicin. OD600 measurements reveal a systematic lag phase elongation with antimicrobial exposure comparable to the heat experiments. OD600 MIC intervals for ciprofloxacin and ampicillin are consistent with those derived from the corresponding heat flow measurements and overlap with the EUCAST range. The OD600-based interval for gentamicin 0.55 mg/L < MIC ≤ 1.1 mg/ also overlaps well with the EUCAST range (Table 1), in contrast to the heat measurement result (Fig. 4h). In (a), (b) and (c), OD600 values of bacterial growth under antimicrobial stress decrease gradually with increasing drug concentration. All curves represent mean ± SE (n = 3). “MH” in the graphs corresponds to pure medium without bacteria.

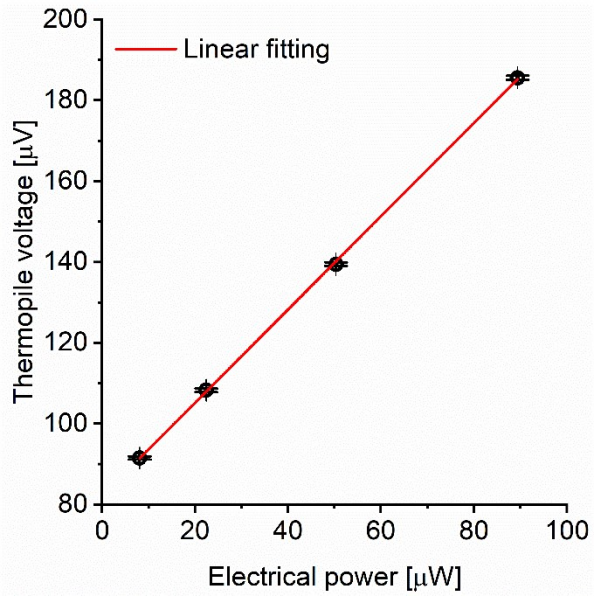

Fig. S6. Calibration of the heat power/thermopile voltage conversion coefficient. The conversion coefficient was obtained by means of an Al thin-film resistor deposited on the sensitive part of the thermopile chip membrane (see Fig. 1c and 1d). A power/voltage conversion coefficient of 0.88 W/V was determined by linear regression of the thermopile voltage output vs the applied electrical power (n = 3). Calibration was carried out with a deionized (DI) water-filled microincubator positioned on the sensor membrane.

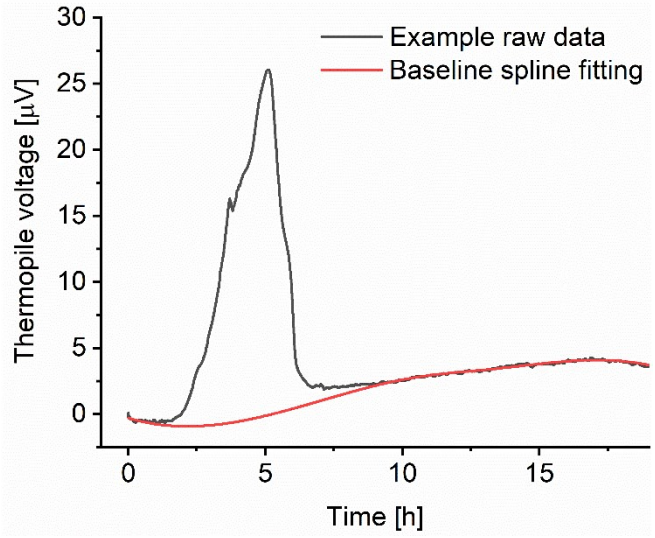

Fig. S7. Heat flow baseline correction. The Fig. shows a representative example for the baseline correction applied in the present experiments. The black curve represents the raw

data of a heat flow measurement (voltage signal in μV). A baseline spline fit (red curve) was generated by OriginLab® software based on 3 separated points in the initial flat region and 3 points in the region after 11 h. The raw data is corrected by subtracting this baseline.

Table S1. Nutrient content of different culture media*

Brain Heart Infusion medium (BHI)	Mueller-Hinton broth (MH)	Lysogeny broth (LB)
12.5 g/L Brain infusion solids	2.0 g/L Beef dehydrated infusion	5 g/L Yeast extract
5.0 g/L Beef heart infusion solids 10.0 g/L	17.5 g/L Casein hydrolysate	10 g/L Tryptone
Proteose peptone	1.5 g/L Starch	10 g/L NaCl
2.0 g/L Glucose		
5.0 g/L Sodium chloride		
2.5 g/L Disodium phosphate		

*according to data sheets available on *www.oxoid.com* and *www.sigmaaldrich.com*