

Supplementary Information

Antimicrobial susceptibility testing by measuring bacterial oxygen consumption on an integrated platform

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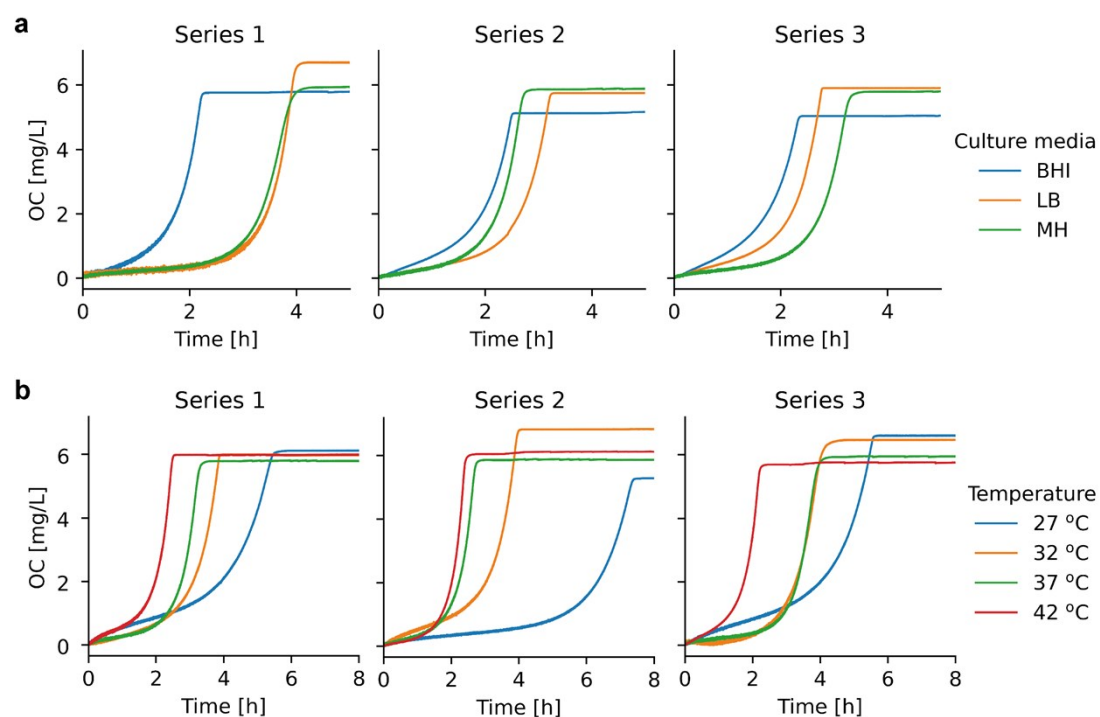


Fig. S1 Oxygen consumption (OC) curves for different culture conditions (3 repetitions of each measurement). (a) OC of bacterial culture in different media: BHI (blue), LB (orange), MH (green). (b) OC of bacterial culture in MH at different temperatures: 27 °C (blue), 32 °C (orange), 37 °C (green), 42 °C (red).

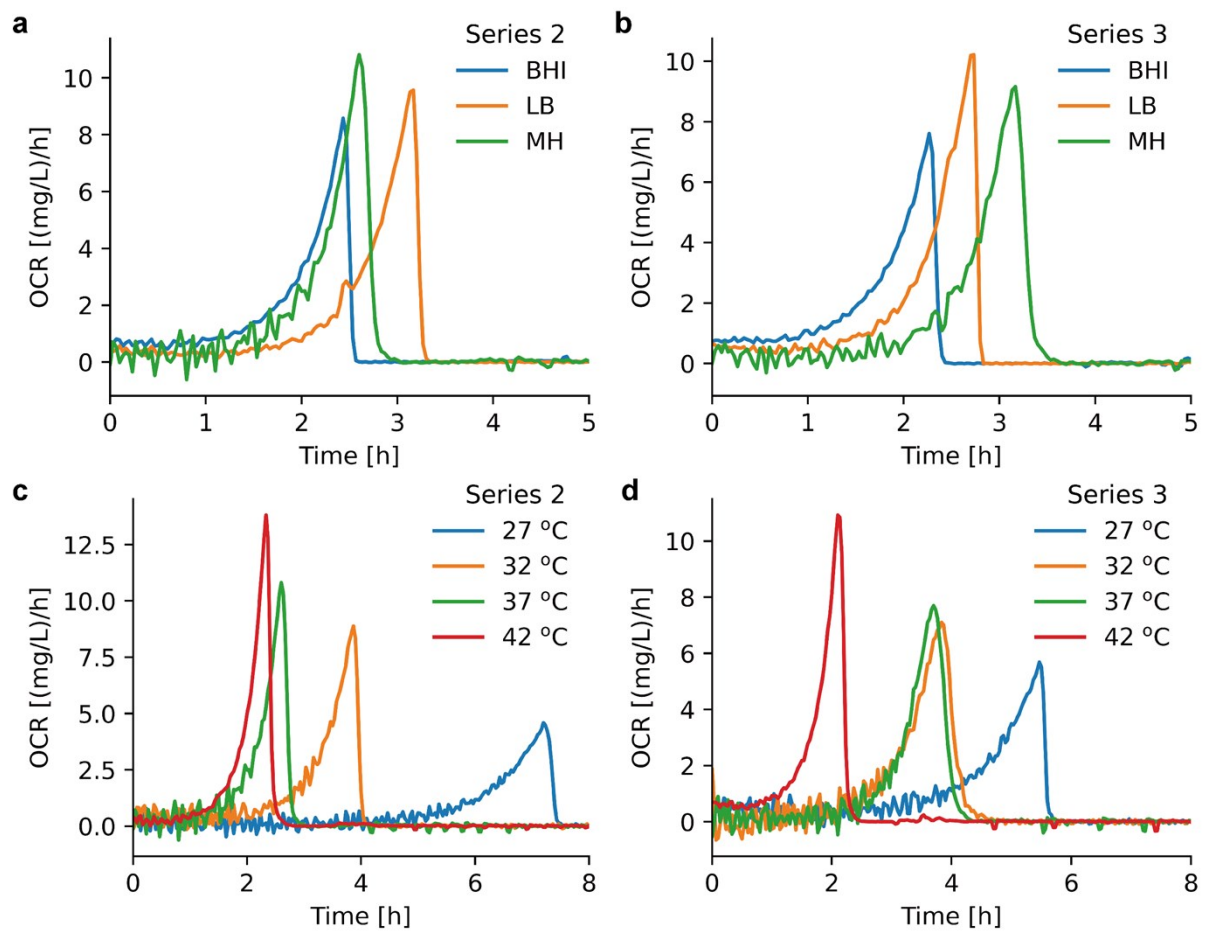


Fig. S2 Additional OCR measurements series for (a, b) different culture media and (c, d) different temperatures. (a, b) Oxygen depletion occurred earlier for BHI, however, overall the impact of the culture media on OCR is subjected to certain variations (as can be seen in the 3 repetitions, including Fig. 3a). We suggest that the initial inoculum size also plays an important role in the experiments. (c, d) OCR varies in a more systematic way as a function of the culture temperature (as derived from the 3 repetitions, including Fig. 3b). Appropriate culture temperatures (42 °C and 37 °C) showed earlier oxygen depletion than the lowest temperature (27 °C).

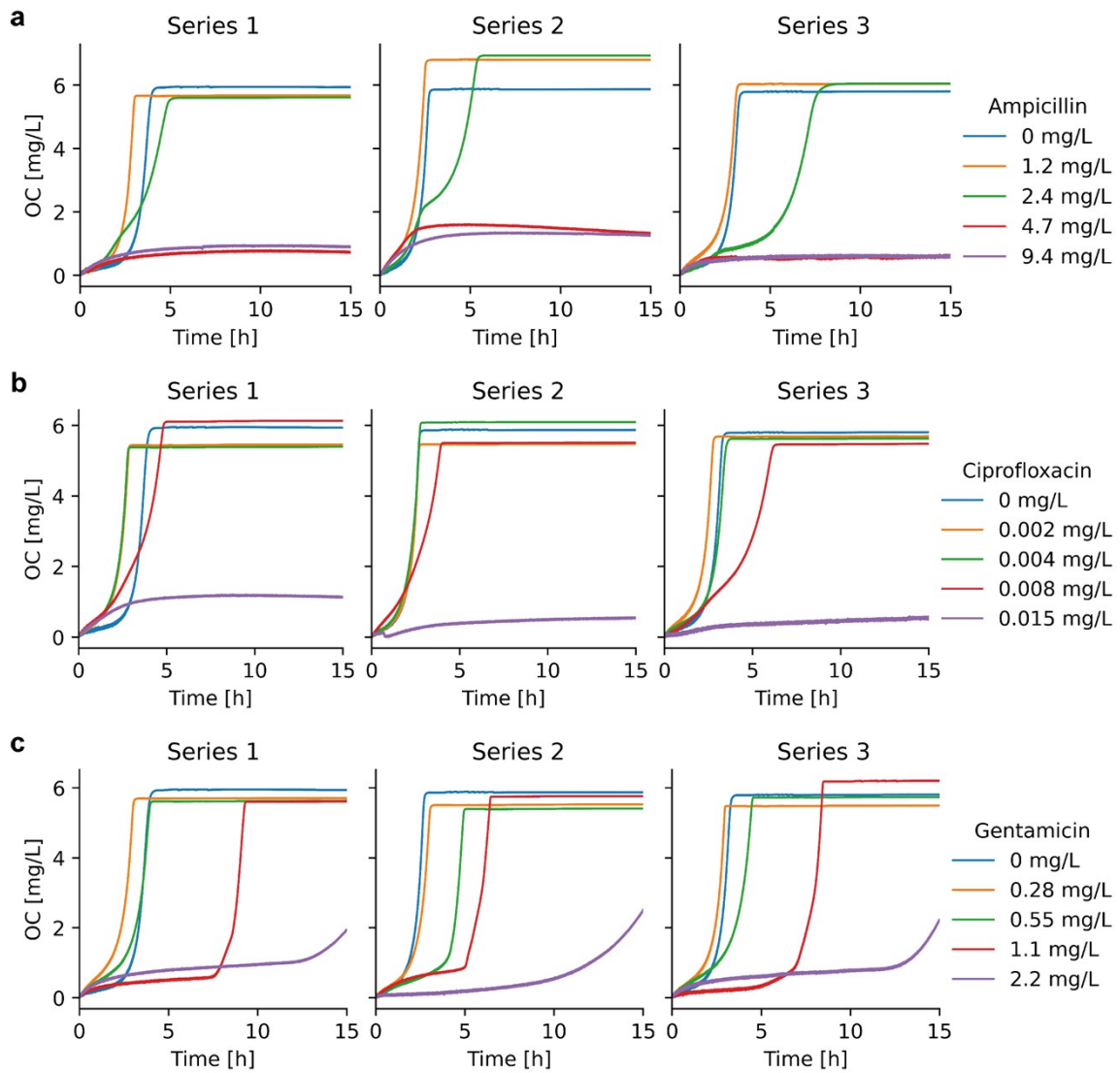


Fig. S3 OC curves for bacterial culture with different antibiotics and a relevant range of concentrations (3 repetitions of each series): (a) ampicillin, (b) ciprofloxacin, (c) gentamicin. OC is maintained close to zero-level for antimicrobial doses higher than the MIC value.

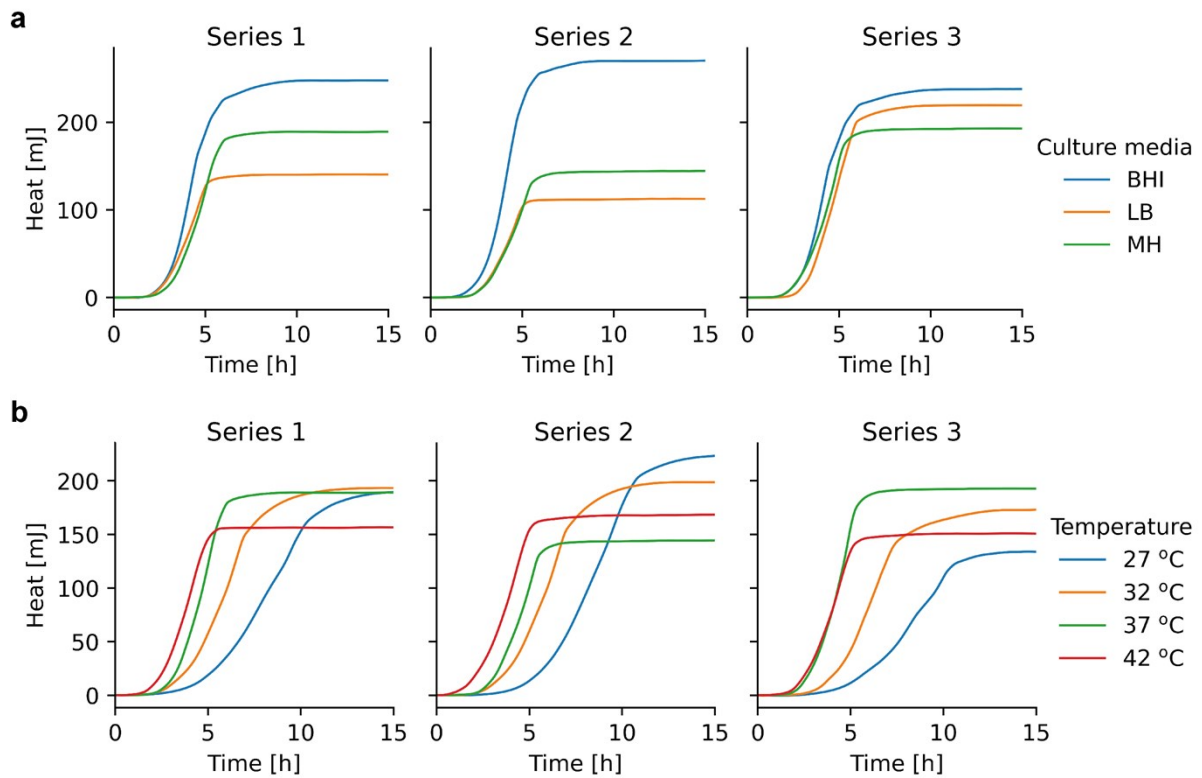


Fig. S4 Metabolic heat curves for different conditions (3 repetitions). (a) Heat production of bacterial culture in different media: BHI (blue), LB (orange) and MH (green). The total heat production increased with the amount of nutrients provided by the medium (BHI > MH ~ LB, see Table S1). (b) Heat production for different temperatures: 27 °C (blue), 32 °C (orange), 37 °C (green), 42 °C (red).

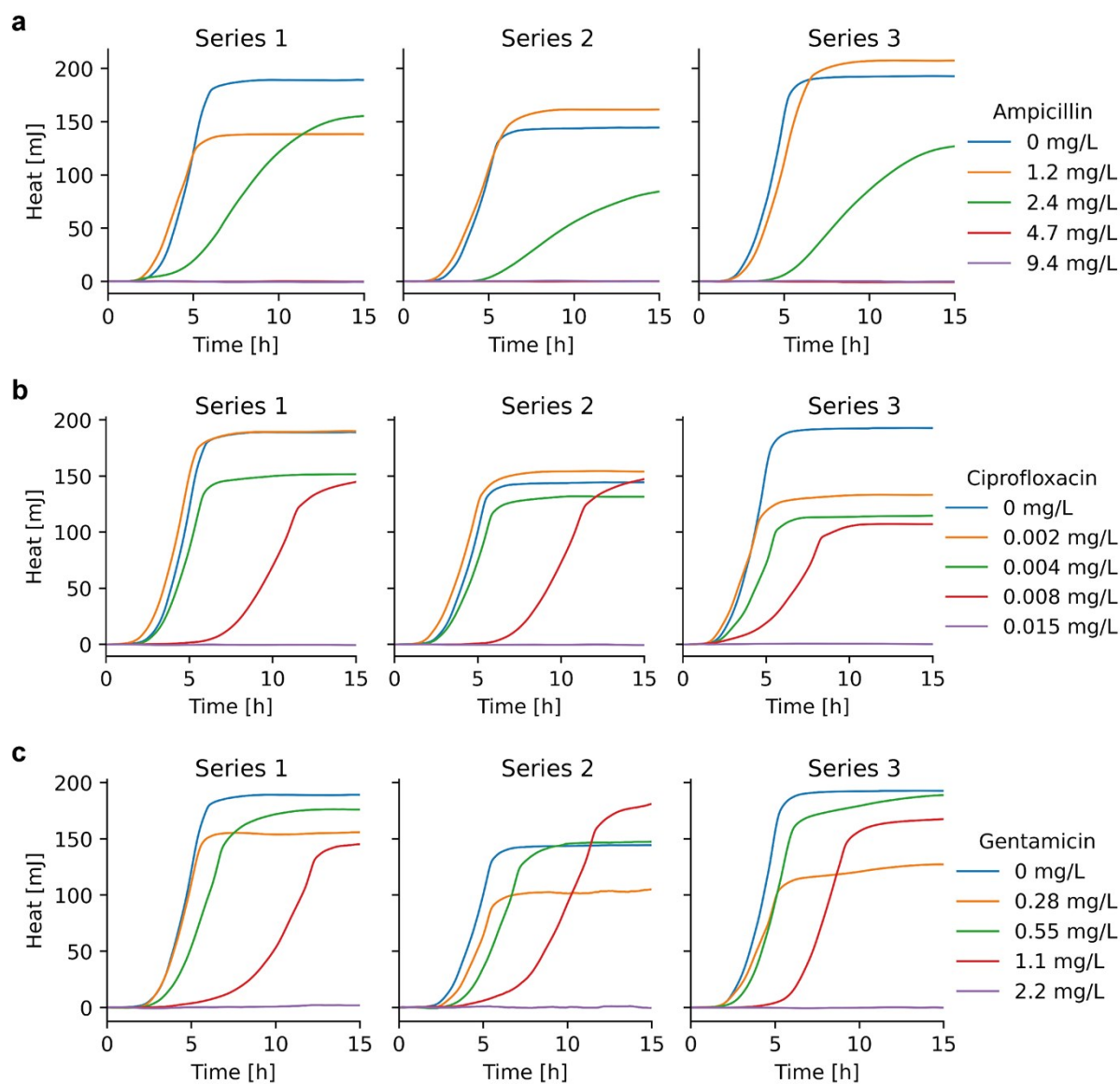


Fig. S5 Metabolic heat for bacterial culture in different antimicrobials (3 repetitions): (a) ampicillin, (b) ciprofloxacin, (c) gentamicin. Heat production is another indicator of the bacterial metabolic activity, which can be applied for AST assays as well. As shown in the different plots, heat production ceased for antimicrobial doses higher or equal to MIC (purple curves).

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		
5.0 g/L Beef heart infusion solids	2.0 g/L Beef dehydrated infusion	5 g/L Yeast extract
10.0 g/L Proteose peptone	17.5 g/L Casein hydrolysate	10 g/L Tryptone
2.0 g/L Glucose	1.5 g/L Starch	10 g/L NaCl
5.0 g/L Sodium chloride		
2.5 g/L Disodium phosphate		

*according to data sheets available on www.oxid.com and www.sigmaldrich.com

Table S2 Minimum inhibitory concentration (MIC) obtained with the OCR platform compared to standard EUCAST* values (*E. coli* ATCC 25922). Our measurements allow defining intervals into which actual MIC values fall. For ciprofloxacin and ampicillin these intervals overlap well with the MIC range established by EUCAST. For gentamicin, the OCR MIC interval is slightly higher, possibly due to reduced activity after oxygen depletion in the microincubator.

Antibiotic	OCR MIC interval (mg/L)	EUCAST MIC range (mg/L)
Ciprofloxacin	0.008 < MIC ≤ 0.015	0.004 - 0.016
Ampicillin	2.4 < MIC ≤ 4.7	2 - 8
Gentamicin	1.1 < MIC ≤ 2.2	0.25 - 1

*The European Committee on Antimicrobial Susceptibility Testing