Combining growth inhibition assays and metabolic profiling to accelerate discovery of antibiotics with unconventional modes of action

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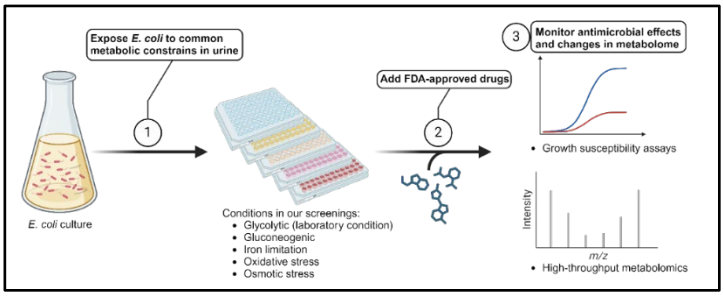
Antibiotics are daily used to treat bacterial infections and as prophylactics in clinics. Despite successful antimicrobial therapies, it is estimated that bacterial infections are responsible for around a third of the global health burden. The increasing antimicrobial resistance in the last decades is a global health challenge, making conventional antimicrobial therapies inefficient. Despite the clear need for antimicrobials, global efforts on antibiotic discovery have significantly declined in recent decades. Furthermore, antimicrobial discovery approaches generally rely on growth inhibition screenings of compound libraries, where pathogens are cultured under optimal growth conditions. This leads to the selection of candidates that might inhibit bacteria growth in the laboratory but not in humans, where the physiology of bacteria is affected by the site of infection (e.g., metabolic constraints). In other words, promising candidates might fail *in vivo*, while others that work *in vivo* might be overlooked due to poor efficacy in laboratory settings. Moreover, screenings solely based on growth susceptibility assays do not provide any insights into the drug's mode of action, leading to a delay in drug characterization.

In this study, we aim to find novel antimicrobials using urinary tract infection as a disease model by screening FDA-approved compounds in *Escherichia coli*. To mimic *in vivo* conditions, we performed the screening in five different growth media with metabolic constraints found in urine. In parallel to growth susceptibility assays, we measured bacterial physiological changes upon drug exposure by high-throughput metabolomics. We found drugs used in diverse clinical indications (not as antimicrobials) that inhibit bacterial growth with increased sensitivity when bacteria were cultured in our prepared urine-like media. Changes in the metabolome provided us with key insights into the drug mode of action of the candidates. We characterize further the mechanism of action of one promising candidate that shows strong antimicrobial activity when bacteria grow under *in vivo* mimicking conditions. Besides, this candidate shows inhibitory effects on biofilm formation in the Gram-positive bacterium *Staphylococcus aureus*.

In summary, our approach serves as a proof-of-concept for developing strategies to screen compound libraries more efficiently and identify molecules with unique antimicrobial modes of action.



Schematical figure: Bacteria is grown under metabolic constraints found in urine to mimic *in vivo* conditions. Screening of around 80 FDA-approved drugs were performed in each medium, and growth inhibitory effects were quantified. Physiological changes in bacteria were determined by high-throughput metabolomics.

Figure created with BioRender.