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Development of a Synthetic Human Urine formulation for the study of uropathogenic *E. coli* (UPEC) physiology and the discovery of antibiotics

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Approximately 150 million people develop a urinary tract infection (UTI) every year and uropathogenic *Escherichia coli* (UPEC) are responsible for ~75% of these infections. Nevertheless, the majority of our knowledge about how antibiotics exhibit their activities is generated from non-pathogenic lab-adapted *E. coli* grown under optimal, nutrient-rich growth conditions. However, UPEC strains are metabolically and physiologically adapted for growth in the harsh environment of human urine, which can have dramatic impact on the susceptibility towards certain antibiotics. Real urine varies a lot in its composition, therefore we aimed to create a synthetic human urine formulation in which the metabolic behavior, the nutritional limitations and the stresses that UPECs face in real human urine is reflected in an axenic, highly reproducible growth medium.
We applied nuclear magnetic resonance (NMR) and mass spectrometry (MS) to detect the compounds consumed by UPECs during growth in human urine and supplement these metabolites to an axenic media with human urine characteristics.
The growth dynamics of over 100 uropathogenic and environmental *E. coli* in the newly developed synthetic urine closely reflected the growth dynamics in real urine. Additionally, we applied proteomics to benchmark the cellular program of UPECs grown in the synthetic urine versus real urine. We compared the minimal inhibitory concentration (MIC) of standard-of-care antibiotics for UPECs in rich growth media vs. pooled and synthetic urine. Our results demonstrate a 250-fold lower activity in MHB (and 4000-fold lower in LB) compared to pooled urine for one of the most used antibiotic to treat UTIs. This difference is due to a specific limitation that UPECs face in urine but not in broth and it indicates that other limitations that uropathogenic bacteria experience in urine can be exploited to discover novel antibiotics in synthetic urine that would be overlooked in rich growth conditions.