California Institute of Technology

Rapid Phenotypic Antibiotic Susceptibility Testing in 30-minutes Directly from Clinical Samples via Single-molecule Quantification

Countless lives will be saved and the spread of antibiotic resistance will be halted with a diagnostic test that can rapidly (within a doctor's visit) and robustly identify (ID) the pathogen causal of the disease and measure the pathogen's drug susceptibility to antibiotics—all at the point-of-care (POC). No such test exists today in the clinic or even in the academic literature that can take a clinical sample (especially one with low pathogen load, such as blood) and directly identify the pathogens and perform antibiotic susceptibility testing (AST) in less than 1 hour. Using technological innovations in microfluidics, chemistry, and microbiology, our team is developing a platform to perform such a test. Our ID approach will identify pathogens rapidly, in less than 10 min from some clinical samples. Our novel AST approach discovers universal markers of antibiotic susceptibility that allow us to (i) differentiate susceptible and resistance phenotypes by analyzing the phenotypic (physiological) response of a bacterium to an antibiotic, and (ii) get a phenotypic readout directly from a clinical sample that is rapid, pathogen-specific and ultrasensitive. To achieve these capabilities, we use digital, single-molecule amplification to quantify nucleic acid (NA) markers that reflect changes in a pathogen's DNA replication or RNA expression upon exposure to antibiotics (ABX). We have already validated the theoretical foundation of this digital AST (dAST) platform. We have shown that our dAST works with diverse antibiotic classes, including beta-lactams (Schoepp et al. *Angew Chemie* 2016, doi: 10.1002/anie.201602763) and it can be applied directly to clinical samples, such as urine from patients diagnosed with urinary tract infections (UTIs). We have also shown that our entire AST workflow, from a clinical sample to answer, can be performed in less than 30 min and is thus amenable to POC settings. We will further develop and validate the dAST approach for use with high priority pathogens (including the most "urgent" and "serious" threats on the CDC's priority list) and a range of sample types (including blood), and we will develop integrated devices that will automate the entire workflow of pathogen ID and AST. Such devices will make the ID and dAST processes user-friendly, robust, safe, and appropriate for CLIA waiver and global distribution. Our novel dAST approach sets the foundation for a new generation of rapid AST diagnostic devices that will ultimately improve therapeutic decisions, accelerate clinical trials for the development of new antibiotics, and enable antibiotic stewardship.