30 Minute Pathogen-specific Phenotypic Antibiotic Susceptibility Test Directly From Clinical Samples Using Digital LAMP Quantification

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Rapid antimicrobial susceptibility testing (AST) would decrease misuse and overuse of antibiotics. Genotypic tests, ones that look for a particular mutation or gene known to be associated with resistance, can work for gram positive organisms but are more difficult to deploy for gram negative organisms where over a thousand resistance genes are known for penicillin alone. Phenotypic tests that evaluate the phenotype of a response when bacteria are exposed to the antibiotic of interest are more attractive, but have been very slow because they required long incubation of bacteria with antibiotics. The "holy grail" of AST is a phenotype-based test that can be performed within a doctor visit (30 min.). To achieve this requires determining a pathogen's susceptibility after a short antibiotic exposure. We used digital PCR (dPCR) to precisely measure DNA replication in bacteria exposed to antibiotics, which shortened the required antibiotic exposure time by allowing very high resolution quantification of DNA replication on timescales faster than cell division. Partitioning bacterial chromosomal DNA into many small volumes during dPCR enabled rapid AST via (i) precise quantification and (ii) a measure of how antibiotics affect the states of macromolecular assembly of bacterial chromosomes. This digital AST (dAST) determined susceptibility of clinical isolates from urinary tract infections (UTI) after 15 min of exposure for all four antibiotic classes relevant to UTIs (*Angewandte Chemie*, Schoepp *et al.* 2016). We then optimized the chemistries and performed the entire dAST workflow directly from clinical UTI samples in less than 30 min. This work lays a foundation for the development of a rapid, point-of-care AST that would improve patient outcomes and strengthen global antibiotic stewardship.