Microfluidics for Rapid Antibiotic Susceptibility Testing:

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The issue:

Antibiotics have been an essential part of healthcare since the early 20th century and has been effectively used for the prevention and treatment of infectious disease. However, the extensive and indiscriminate use of broad spectrum antibiotics has led to the rise in antibiotic resistance strains of bacteria. The threat posed by super bugs or multidrug resistant bacteria are real and is not only predicted to affect the developing nations but is a global threat leading to a 'post-antibiotic era'. This has led to several known antibiotics being ineffective to the pathogen causing the infection thus once-preventable disease becomes infectious again. It is imperative, now, to understand antibiotics, and their resistance profile before prescribing appropriate antibiotic rather than broad-spectrum. This is possible only is the antibiotic susceptibility tests (AST), in which the resistance level of bacteria to a drug is evaluated, are rapid, sensitive, cost-effective and quantitative.

Current Solutions and their issues:

Current gold-standards of AST include broth-dilution, disk-diffusion, stripe tests for plate cultures which rely on the growth of bacteria to countable colonies in agar plates or to a certain measurable turbidity in liquid cultures. This typically takes > 16 to 24 hrs which is increased depending on the type of bacterium causing the infection and its resistance profile. Novel AST methods are on the rise in recent times and can be grouped into genotypic methods such as PCR-based resistant gene detection, genome sequencing, mass-spectrometry, microarrays which however has limited utility since only few genes ae associated with phenotypic antibiotic resistance which leaves newly acquired mechanisms undetected; and phenotypic methods include monitoring cell growth – size and morphology variation at both the single cell level as well as a culture, analysing their viability, growth rate, metabolic molecules and/or antibiotic degradation products, which are used as indicators and read out by electrochemical, optical (fluorescent, turbidity, colorimetry), bioluminescent, nanomechanical means.

These emerging novel phenotypic ASTs are typically on microfluidic platform which are scaled down analogue of the culture flasks, mimicking the microenvironment in addition to providing a window for monitoring cell growth and dynamics at single cell level, broad range of approaches, tools and new routes to AST and antibacterial resistance testing.

Proposed solution

I would like to propose a droplet microfluidic approach for AST where each droplet acts as a nano-liter microreactor providing the essential nutrients for growth of bacteria. Growth in a microdroplet is known to be accelerated due to the improved mixing within each droplet as well as due to the small volume of the sample; this typically opens up the possibility of tracking the turbidity or the optical density of the culture in the droplet within few hours (< 2 hrs). I would like to pursue this and monitor the variation in the growth and turbidity indicator, plot standardized growth curves, with and without antibiotics. I also propose to track the turbidity in the microdrop, using a LED and a photodiode to convert the optical signals to measurable electrical signals. Once standardized, a comparison of the clinical samples with these standard curves, will provide the antibiotic susceptibility and resistance profile of the clinical pathogen withing the timeframe thus leading the correct prescription.

Work plan

The work plan is divided into 2 phases.

Phase 1 is the initial research work in MicroReaction Engineering Lab to be completed in 1.5 years. Phase 2 will be performed based on the inputs from phase 1 and depending on a clinical collaboration.

Phase 1

1. Development of droplet microfluidic module: A cost-effective droplet microfluidic module will be developed either based on glass capillary or 3d printing. Formation of nanoliter droplets will analysed and the best module will be considered for further processing. The key parameters for evaluation will be – throughput, ease of handling and monitoring and cost.
2. Standard cultures in the droplet: *E coli* (DH5a) will be cultured in the droplets and their growth rate will be monitored under standard conditions. The drops will be docked inside capillary tubes maintained at 37 C, over a period of time and the growth inside the drops will be monitored colorimetrically. We will be using resazurin dye for this purpose, which changes color due to the change in pH caused by the metabolites released during growth. Other dyes such as fluorescein will also be tested if necessary. The parameters tested will be – size of the droplet, flow rate in the channel, initial inoculum concentration and time for colour change.
3. Automated monitoring: We will also be working on integrating the LED and the photodiode with the capillary tube and monitoring the color change/turbidity change. LabView programs will be used to obtain the essential data.
4. Antibiotic susceptibility testing (AST): Varying concentrations of antibiotics will be introduced into the droplets and the real-time color change/turbidity change will be monitored and analysed over time. This will be compared to the results in step 2 to determine the susceptibility or resistance of the pathogen to the antibiotic.

Phase 2: Future scope

- The developed method is for a single AST. This can be parallelized and extended to multiple ASTs.
- The proposal is specifically for *E coli,* which can be extended to several other pathogenic species of bacteria and for multiple antibiotics.
- The proposal can be integrated into a diagnostic platform and can be used for clinical samples – specifically for UTIs, which are the primary cause of brod-spectrum antibiotic prescriptions and therefore antimicrobial resistance strains development.

Deliverable:

Phase 1: Standardized conditions and the potential use of droplet microfluidics for AST.

Phase 2: A simple POC device which can be used in hospitals for rapid AST.

Budget

S.No.	Type	List	Cost (INR)
1	Microbiology consumables	LB agar, LB medium, plates, ATCC bacteria, antibiotics, glass ware	2 L
2	Microfluidic consumables	Glass capillary, capillary tubes, external Tees, connectors, 3d printing polymer, chip fabrication polymer, oil (silicone oil and fluorinated oil), surfactant, transparent tubes for docking drops	10 L
3	Equipment	Syringe pump (Harvard apparatus for low flow rates ~ 0,01 µL/min)	4.5 L

4	Consumables for readout	Dyes (resazurin, fluorescein), LEDs (several wavelengths from 500 to 700 nm), photodiode	1.5 L
5	Facility usage/contingency		1L
6	Overheads	5%	1L
		Total	20 L