Project proposal for Biomaker challenge:

Title of Project: A low cost reusable microfluidic device for the detection of antibiotic resistant genes in bacteria isolated from patient samples.

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Summary:
Modern day biomedicine has been revolutionised by the introduction of molecular diagnostic methods. With recent advancement of high throughput DNA and protein sequencing technologies we are living in the omics era where personalised medicine became a familiar term. Despite significant technological advancement, molecular diagnostic methods are not within the reach of most people in the developing world. For example, antibiotic resistant (Multiple drug resistance or MDR and Extensively drug-resistant or XDR) forms of Mycobacterium tuberculosis, is a raising threat to the world and it is necessary to detect the presence of such resistant strains in patient samples as early as possible. Polymerase chain reaction (PCR) based methods for the detection of antibiotic resistant form of bacteria exist in the market, but use of such technology is not cost effective and often depend on expansive hardware along with continuous purchase of consumables like specific cartridges. Here, in this project we propose to develop a microfluidics based, reusable module, mainly to detect the presence of antibiotic resistant bacterial genes in patient samples.

i) the problem we are addressing:
Emergence of multiple antibiotic resistance in bacteria is one of the major concern of healthcare professionals presently. Antibiotic resistant forms of *Mycobacterium tuberculosis*, causing MDR or XDR TB, have been identified as a global threat by several eminent healthcare agencies like world health organisation[1] or Centers for disease control and prevention[2]. For efficient management of infectious disease like drug resistant TB, it is necessary to detect the presence of the antibiotic resistant form of the bacteria in the patient sputum sample.

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Until now the primary detection of tuberculosis depends on staining of sputum smear and detecting the presence of bacillus bacteria (Acid fast bacilli) in the patient sample, a method developed almost a century back[3]. Tuberculin skin test is another way of detecting the presence of reactive antibody in patient body. Chest X-ray is also deployed when lung infection is suspected. But deploying any of these above-mentioned methods, it is not possible to detect the antibiotic sensitivity of the infecting mycobacteria. Presently the antibiotic sensitivity is detected by culturing the mycobacteria present in sputum sample, but this is a time-consuming process and takes up to 4-6 weeks' time. This is one of the major reason of therapy failure, as quiet often the patients with antibiotic resistance TB are given regular course of antibiotics, (Rifampicin, Isoniazid, Pyrazinamide, and Ethambutol, which are considered as first line of defence) before knowing the nature of antibiotic sensitivity. Other than serious risk of therapy failure, the 4-6 week delay in detecting the antibiotic sensitivity often allow the antibiotic resistant form of the disease to spread. Recently a nucleic acid amplification based assay has been developed to detect Rifampicin resistance, which is performed in a fully-automated platform called Xpert MTB/RIF[4]. The Xpert system depends on polymerase chain reaction (PCR) based amplification of Rifampicin resistance gene in the bacteria present in the patient sample, followed by fluorescent detection of the amplified gene product. Despite its fully automated mode of action and fast detection time, the Xpert system depends on an expansive assay-detection hardware and the use of single use cartridge for specific antibiotic resistance mutation detection [5], thus it has a high recurring running cost, which is a problem for developing countries to afford.
To approach this problem, here we propose to develop a microfluidics based nucleic acid amplification system to amplify the antibiotic resistance gene from the bacteria present in patient samples. We further propose to detect the amplified nucleic acid product, by using a fluorogenic substrate and a LED array.
During the past decade Microfluidic PCR devices have been developed, but not been used to address the specific problem we are addressing [6]. These recent developments propelled our thought couple microfluidic based PCR system with LED based optical detection method, to develop the proposed device.

ii) the biological systems we are using:

To test our device, we are planning to use synthetic gene, and if possible non-pathogenic *E. coli* bacteria at the end. This will be sufficient to test the nucleic acid amplification ability of our device, which could further be extrapolated to the patient samples.

iii) the design goals for the hardware:

The proposed device will have two main sections (Fig1). The first section will be the microfluidic chamber, where the bacterial sample and nucleic acid amplification reagents will be injected. A thermal array will be deployed to carry out the thermal cycling based nucleic acid amplification. After nucleic acid amplification, the product could be drawn into an optically clear chamber where the amplified product could be detected using a LED and photodiode array. Using an array would allow for multiple fluorescence makers to be screened for within a single sample, by staggering the LEDs. Depending on the sensitivity of the photo diodes used a lens or filter may be required to reduce the signal to noise ratio.
Thermal cycling, the movement of fluids and LED switching, will be controlled with the aid of an Arduino.

iv) how you plan to implement the project:

After developing the device, we are planning to test it extensively using synthetic genes, and non-pathogenic bacterial strain.

v) the proposed outcomes and benefits:

Our proposed device will have several benefits, which are listed below:

a. Low running cost, and low recurrent expenditure: Our proposed device is based on a microfluidic platform, and could be used multiple times. This device will eliminate the need of using specific cartridges for the detection of different mutations.
b. The proposed device could be deployed extensively for molecular diagnosis, and could be used to detect a wide array of genetic variants ranging from single nucleotide

b. The proposed device could be deployed extensively for molecular diagnosis, and could be used to detect a wide array of genetic variants ranging from single nucleotide polymorphisms (predicting disease susceptibility), presence or absence of multiple antibiotic resistance genes, using specific oligonucleotide primers. It is noteworthy to mention that multiple antibiotic resistant genes or point mutations conferring antibiotic resistance have been revealed recently by extensive research[7,8] . The proposed device could be used to detect the presence of such antibiotic resistant alleles.

c. This device can effectively be battery powered, thus can be used effectively in remote locations and efficiently in mobile healthcare facilities.

vi)Estimate the components and budget that we need to complete the project:

Component	Purpose	Cost
LED (λ=395nm)	GFP stimulation	≈£1
Photodiode(λ=540nm)	GFP-fluorescence detection	≈£1
F-plate heater 10W	For heat cycling	≈£20
Peristaltic Pump	Flow control	≈£30
Arduino UNO	System control	≈£20

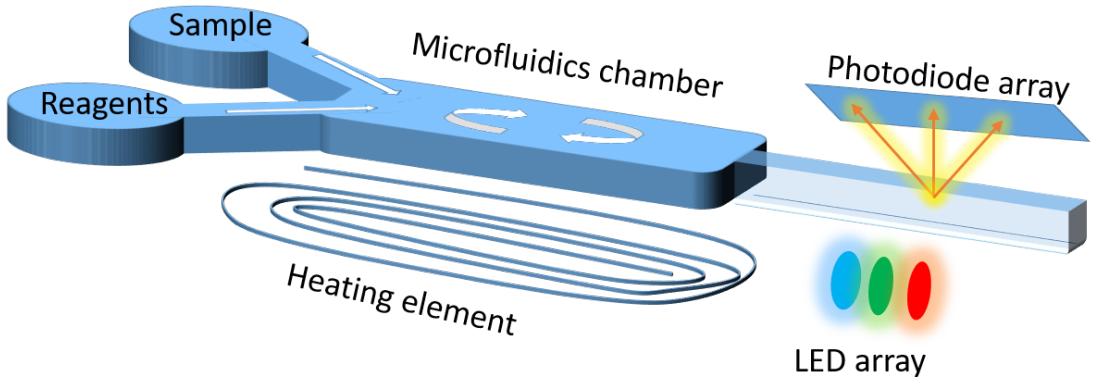

Figure 1 Proposed device excluding controllers, pumps and other circuitry.

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