Laser speckle imaging system for evaluation of antimicrobial resistance

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Abstract
Laser speckle imaging is a method that applies reflected laser light, creating a speckle image when it is reflected off a surface. The changes of the speckle image can be tracked to determine movement of very small objects on the surface such as cells and bacteria. It has also been demonstrated that laser speckle imaging is an improved method of bacterial growth measurement over traditional laboratory methods [1]. Preliminary measurements were conducted at Pauls Stradins Clinical University Hospital Joint Laboratory using *Enterobacter cloacae* Piperacilin-Tazobactam at 36mg/L concentration to test if the formation of a sterile zone while using the disk diffusion method can be detected earlier than using the standard method.

Introduction
Bacterial resistance due to widespread of use of antibiotics is a serious problem for modern medicine as common bacterial species have become insensitive to traditionally used antibiotics. In case of infection, the appropriate treatment must be determined as quickly as possible. This can be achieved by testing the specific pathogen for susceptibility to several antibiotics at the same time in a laboratory. Recently, this issue has become relevant in the treatment approach of COVID-19 patients as severity of disease is strongly related to presence of bacterial coinfection [2]. Finding the effective antibiotic treatment quickly is crucial to reduce mortality of patients and to avoid the formation of new antibiotic-resistant bacteria due to preventative antibiotic use. Currently utilized “golden standard” methods, namely, disk diffusion and E-test require 16-24 hours to conduct [3]. Laser speckle imaging could make this process faster and more precise.

Methods and Materials
Preliminary experiments were done on *Enterobacter cloacae* in the Pauls Stradins Clinical University Hospital Joint Laboratory. The bacterial sample was provided by the laboratory. The bacterial colony was prepared for antibiotic resistance testing according to EUCAST standards. The antibiotic used was Piperacilin-Tazobactam at 36mg/L concentration.The specific antibiotic was chosen due to known sensitivity of the bacteria to it [4].

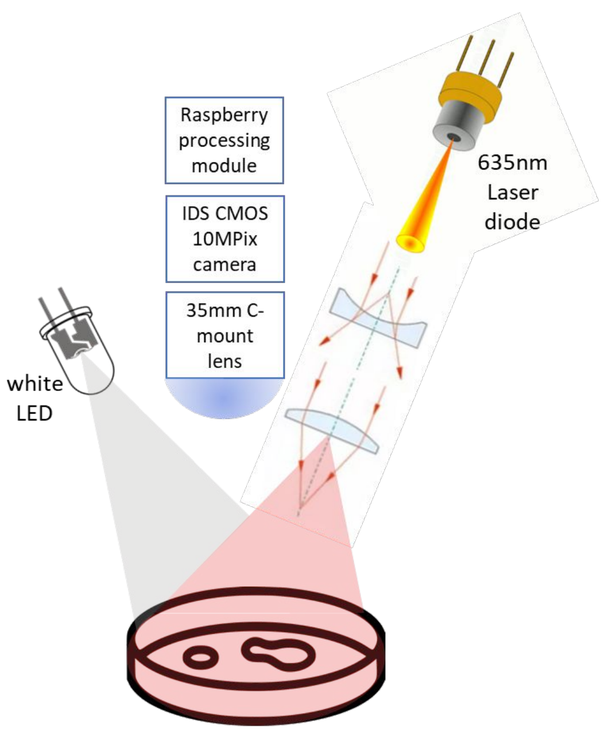

Figure 1: Laser speckle imaging device consisting of 635nm laser diode and white LED for illumination of the sample, IDS CMOS 10MPIx camera, 35mm objective lens and a Raspberry processing module for capturing imaging data

The petri dish containing the live bacterial sample and the antibiotic where then put in a thermal oven for 10 hours at 36^{o}C together with the laser speckle imaging device. The design of the device is shown in Figure 1.

An image was captured at 30 second intervals. The images were then processed by dividing the experimental field into small sections of NxN pixels. The following steps were performed for each such section separately.
A two-dimensional normalized correlation was performed between consecutive NxN image fragments throughout the experiment [5]:

$$Corr(u,v) = \frac{\sum_x \sum_y ((a(x,y) - \bar{a}) * (b(x-u, y-v) - \bar{b}))}{\sqrt{\sum_x \sum_y (a(x,y) - \bar{a})^2 * \sum_x \sum_y (b(x-u, y-v) - \bar{b})^2}}$$

Where a(x,y) and b(x,y) are two adjacent frames in a sequence, $\bar{a}$ and $\bar{b}$ are the average values of these two frames, u and v is spatial displacement between frames a(x,y) and b(x,y) in the directions of x and y, respectively.
The correlation peak shift in space characterizes the changes that occur between consecutive images. To find a more accurate offset, it is necessary to interpolate around the peak of the correlation. The offsets obtained between each pair of adjacent images were accumulated. Thus, the sequence of images was converted into a “time signal”. To avoid the influence of local transient spikes, it is worth smoothing the signals [6].

Results and Discussion

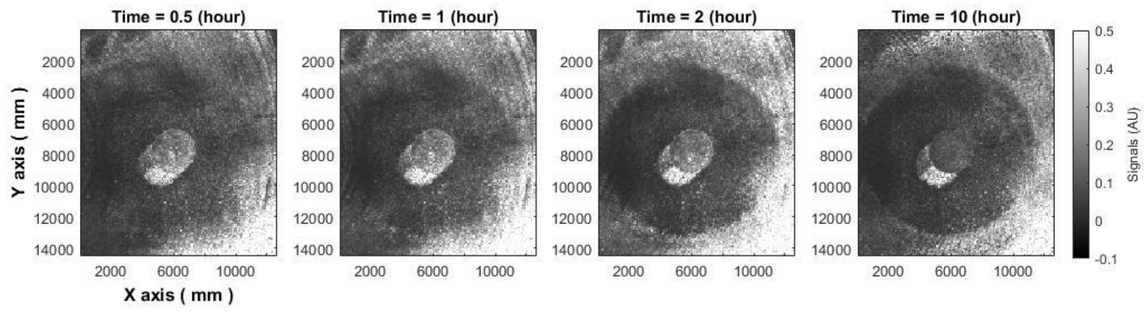

Figure 2: A disk forming in the layer of Enterobacter cloacae around the antibiotic (Piperacilin-Tazobactam, 36mg/L) tablet.

The processed results are in Figure 2. Since most *Enterobacter cloacae* is sensitive to Piperacilin-Tazobactm, a disk forms in the bacterial layer around the antibiotic during the 10 hours of measurement. The formation of the sterile zone is already starting from 0.5 hour and becomes completely visible 2 hours after the start of the experiment. In comparison, according to EUCAST disk diffusion method for this bacterium it takes 18±2h to determine such a result [7].
Future experiments will be conducted using other common infection causing bacteria using antibiotics with different sensitivity. The image capture time will also be reduced to analyse the movement of bacteria.

Conclusions
Preliminary results show that the sterile zone between the *Enterobacter cloacae* bacteria and the Piperacilin-Tazobactam, 36mg/L antibiotic tablet does not change and is detectable after 2 hours.

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