DYNAMIC LASER SPECKLE IMAGING FOR FAST EVALUATION OF THE ANTIBACTERIAL SUSCEPTIBILITY BY THE DISC DIFFUSION METHOD

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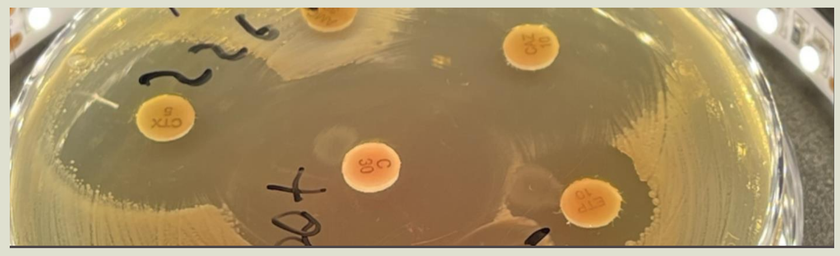
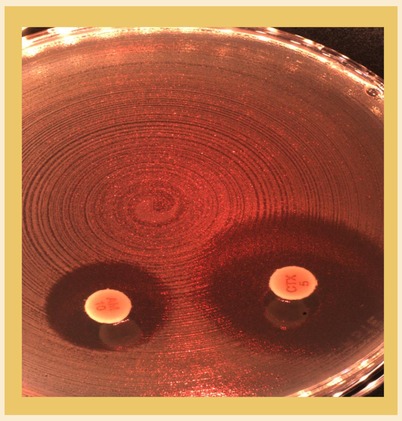


ABSTRACT

Phenotypic resistance tests e.g., disc diffusion method require 16-24 hours to obtain the results, while the PCR tests provide only genotypic type of antibacterial resistance. New methods are needed to assess antibacterial resistance faster than existing methods, thus providing targeted pharmacological intervention at the early stage of the disease, increasing a patient's survival chances. Laser speckle imaging technique allows tracking moving particles in optically inhomogeneous media, allowing to observe the processes of changing the behavior of the sterile zone. The choice and adaptation of a mathematical model describing changes in the growth diameter of the sterile zone as a function of time and comparison with experimental data will help to understand and describe the formation of the sterile zone. The aim is to create processing algorithms for the obtained images to predict the diameter at the end of the disc diffusion test from the diameter of the earliest visible sterile zone.



INTRODUCTION

➔ Early identification of pathogen and its susceptibility to antibiotics can provide targeted pharmacological intervention at the early stage of the disease, increasing a patient's survival chances.

➔ The issue has become relevant in the treatment of elderly patients, immunosuppressed patients, and patients with secondary bacterial complications.

➔ Antibacterial therapy based on the results of laboratory tests, on the one hand, helps to reduce patient mortality, and on the other hand, the spread of resistant bacteria in the human and animal population.

➔ Phenotypic resistance tests such as disk diffusion and E-test require 16-24 hours to obtain the results [1], while the PCR tests provide only genotypic type of antibacterial resistance.

➔The development of new cost-effective methods for evaluation of microbial activity to reduce detection time is a potential field of research.

➔This study compares the mathematical model of growth of the diameter of the sterile zone with experimental results. This will help to understand the behavior of the growth curves of the sterile zone and in the future will allow us to predict the subsequent change in the diameter of the sterile zone based on short-term experiments.

CONCLUSIONS

In the current study, the ability of finding experimental growth curves of sterile zones using sub-pixel correlation analysis of laser speckle images was demonstrated.

The accuracy of the proposed mathematical model of growth curves for sterile zones has been verified in comparison with experimentally obtained curves (using sub-pixel correlation analysis of laser speckle images). The approximation, fitted to our case, describes well the experimental curves obtained. For each sensitive bacterium, to determine the radius of the sterile zone early, these curves will be different, and it is necessary to find the set of parameters for each bacterium against each antibiotic. In the case of resistant bacteria, these curves might be unpredictable and then approximations will not work here.

Thus, the effort put into the determination of these parameters will contribute to the development of an algorithm that allows predicting the change in diameter of the sterile zone around the antibiotic disk much earlier than using the standard disk diffusion method.

MATHERIALS AND METHODS

THE EXPERIMENTAL SETUP

A 658 nm diode pumped solid state laser (output power 60 mW) was used for speckle generation. Images were captured with 20-second intervals by a CMOS camera (Fig.1).

MICROBIAL STRAINS AND CULTIVATION CONDITIONS

The clinical isolate of *E. coli* and antibiotic-Ciprofloxacin disc (5 μg) was chosen for the experiment. The experiments were performed in an incubator at 37°C. The suspension of the *E. coli* was made in saline to the density of a 0.5 McFarland turbidity standard. Culturing on Petri dishes was prepared according to an EUCAST standard procedure [2].

LASER SPECKLE IMAGE CONVERSION TO TIME SIGNALS

The images captured at 20 second intervals were processed by dividing the experimental field into small sections of NxN pixels.

1) A two-dimensional normalized cross-correlation was performed between consecutive NxN image fragments throughout the experiment [9].
2) The changes that occur between consecutive frames were found by the offset at the location of the correlation peak. To find a more accurate value of the offset, the interpolation was performed.
3) Offsets obtained between each pair of adjacent frames were accumulated, converting into a time signal that can be analyzed.
4) To avoid the influence of local transient spikes, a signal envelope within a certain window was used [3].

An increase of signal values will occur when there is an activity increase (due to bacterial growth), and a decrease in signal occurs either due to nutrient depletion or due to the antibiotics action.

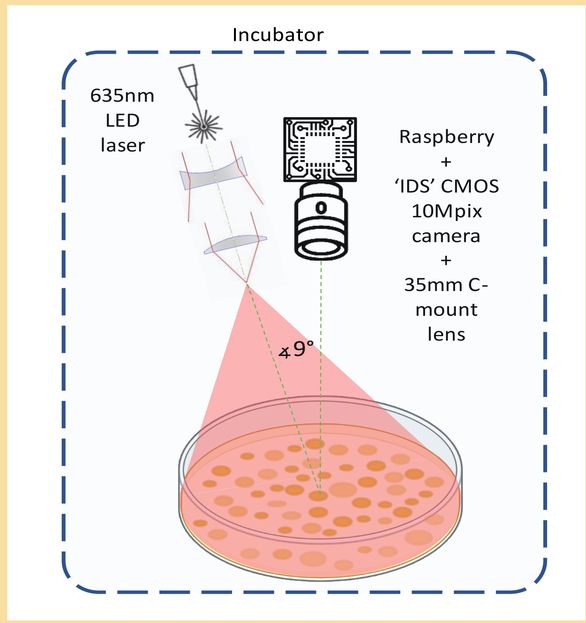


Fig. 1. Setup scheme for burst image capturing of bacteria growing process under 658 nm laser and white LED illumination.

RESULTS

THE ANALYSIS OF STERILE ZONE

A disc of the sterile zone forms around the antibiotic, changing over time. The signal envelopes over each radius were averaged (Eq. 3), to avoid the influence of noise.

$$\overline{Env[r,n]} = \left[\frac{1}{M_{R1}}\sum_{m_{R1}=1}^{M_{R1}} Env_{m_{R1}}[n], \ldots, \frac{1}{M_{Rk}}\sum_{m_{Rk}=1}^{M_{Rk}} Env_{m_{Rk}}[n]\right] \quad (3)$$

Where M_{Rk} is the number of envelope signals at a given radius from the center.

A spatial-temporal behavior of the sterile zone can be obtained by putting all the averaged signal envelopes for all radii together (Fig. 2). Consider the same bacterium: E. coli, and the same antibiotic: Ciprofloxacin, but for two different cases: 1) the antibiotic is placed immediately after the bacteria (almost simultaneously); 2) the antibiotic is placed 4.3 hours after the bacteria. Our previous work describes this situation [4]. The sterile zone can be observed only at certain bacterial activity. 4 h after inoculation the bacteria have grown to a sufficient concentration to demonstrate a signal from bacterial activity. Putting of antibiotic discs 4-5 h after bacterial inoculation allows us to immediately observe the formation of the sterile zone (case 2). Otherwise, the sterile zone will be observed only 3-5 hours after the start of the experiment (case 1).

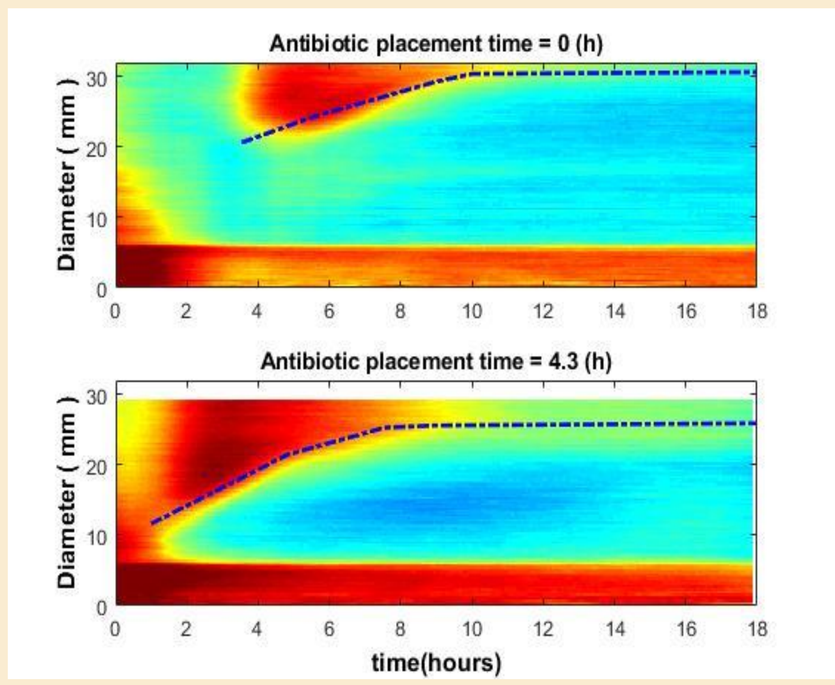


Fig. 2. The growth of E. coli around the Ciprofloxacin disc (5 µg). Spatial-temporal changes of the sterile zone. 1) The antibiotic is placed immediately after the bacteria (top). 2) The antibiotic is placed 4.3 hours after the bacteria (bottom).

THE MATHEMATICAL MODEL OF CHANGES IN THE STERILE ZONE

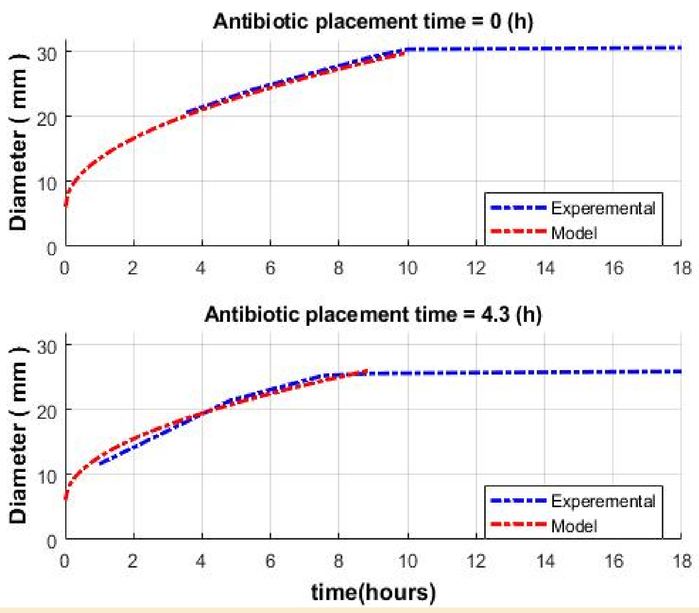


Fig. 3. Spatio-temporal curves for experimental data and model for E. coli around the Ciprofloxacin disc (5 µg).

The minimum inhibitory concentration (MIC) in solid media is usually determined using the agar diffusion technique [5]. Antibiotic diffusion into the agarose medium leads to inhibition of bacterial growth and to the formation of sterile zones. The diameter of sterile zones increases with increasing antibiotic concentration. It is possible to obtain a model of sterile zone radius growth as a function of time [6]. The radius of sterile zone is proportional to the root square of the difference between natural logarithm of the antibiotic concentration c, and natural logarithm of the MIC, multiplied by the diffusion coefficient and the time of antibiotic diffusion (Eq. 4).

$$r = \sqrt[2]{4D(\ln(c) - \ln(\mathrm{MIC})) \cdot \mathrm{t}} \quad (4)$$

where c is antibiotic concentration, and D is the diffusion coefficient received from Fick's second law of diffusion [14] (Eq. 5).

$$\frac{\partial c(x,t)}{\partial t} = D\frac{\partial^2 c(x,t)}{\partial x^2} \quad (5)$$

Fick called this coefficient: "a constant dependent upon the nature of the substances." Parameter c(x,t) describes the dependence of antibiotic concentration on time and on distance from the source. It was assumed that D is independent of concentration [6, 7]. Using this model, we have three unknowns: MIC, D, and c. Their total influence in the formula can be replaced by one constant. Thus, a simple relationship is obtained between the radius of the sterile zone and time (Eq. 6).

$$r = \sqrt[2]{\mathrm{const} \cdot \mathrm{t}} \quad (6)$$

Having obtained the experimental curves, it is possible to choose the parameter of this constant, at which it will be as close as possible to the model. Then compare how much in this case the shape of the experimental curves corresponds to the shape of the curves from the model. The results are presented in Fig. 3.

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This work has been supported by the European Regional Development Fund project "Rapid assessment system of antibacterial resistance for patients with secondary bacterial infections" (No. 1.1.1.1/21/A/034).