Use of a laser speckle system in the determination of antibacterial susceptibility by the disc diffusion method

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Rapid identification of antibacterial resistance plays a crucial role in treatment of acute infectious conditions, Early identification of infectious agent and its sensitivity to antibiotics can provide targeted pharmacological intervention at the early stage of the disease, increasing patient's survival. The issue has become relevant in the treatment of elderly patients. The empiric antimicrobial treatment must be replaced as soon as possible to reduce the risk of mortality of patients. 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.

In previous studies using laser speckle imaging technique, we managed to determine the bacteria growth after 2-3 hours from the beginning of activity [2]. In the laser speckle imaging method, a laser beam is scattered on a Petri dish where the test bacteria and antibiotic discs are located. The laser speckles reflected from the surface are recorded sequentially in time. A sub-pixel correlation analysis was proposed to detect small changes in the sequence of laser speckle images, and the effects associated with changes in bacterial activity can be observed.

Experiments were performed at the Pauls Stradins Clinical University Hospital Joint Laboratory on different bacteria and their corresponding antibiotics [3].

The images captured at 30 second intervals were processed by dividing the experimental field into small sections of NxN pixels. A two-dimensional normalized correlation was performed between consecutive NxN image fragments throughout the experiment. Interpolation around the correlation peak is performed in order to find a more accurate peak position. The correlation peak shift in space characterizes the changes that occur between consecutive images. The offsets obtained between each pair of adjacent images were accumulated and converted to "time signal" [2].

As a result it was obtained that after a few hours from the start of the experiment a disc of the sterile zone begins to form around the antibiotic. The sterile zone becomes clearly visible within a couple of hours after the beginning of formation. The result was obtained significantly earlier than by the disk diffusion method [4] for the same bacterial species [4]. Creating a spatio-temporal image averaging around each radius as it moves away from the centre (from the antibiotic), we can obtain a certain curve, similar to the letter "S", according to which the size of the sterile zone changes over time (Fig. 1). This behaviour has been observed in several different experiments.

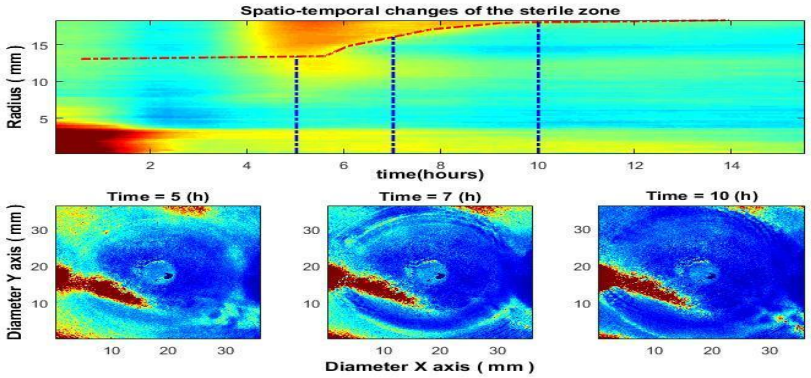

Fig. 1. Spatio-temporal changes of the sterile zone (top), and sterile zone formation in the growth of *Acinetobacter baumanii* around the imipenem disc (bottom).

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