Rapid Antimicrobial Susceptibility Testing Using Laser Speckle Technology

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Abstract - **Antimicrobial susceptibility testing (AST) is key to support clinical decision regarding bacterial infectious diseases treatment. In particular, it is applied to direct the most appropriate antimicrobial therapy to assure its success and thus prevent infection related health complications or even death. In addition, AST is very important to prevent the emergence of bacterial antimicrobial resistance and the spread of multi-resistant bacteria.**

Current standard AST require long periods of time to obtain results, which is an important limitation for the required targeted prescription of antimicrobials. Faced with the challenge of antimicrobial resistance, many proposals have been made to accelerate sample processing, however, the initial step of bacterial incubation prior to AST has not yet been circumvented, being the major contributor to the extremely time-consuming current AST available technologies.

This work presents a new methodology to perform AST, in which the incubation time is reduced to a minimum and the AST process is based on optical technology, the Laser Speckle. Preliminary results of this new AST approach using *Pseudomonas aeruginosa* and *Staphylococcus aureus* clinical strains, two of the most challenging pathogens worldwide, showed very promising results. This new technology may be the future solution to guide antimicrobial prescription, by possibly delivering results in as little as 30 minutes.

Keywords - Laser Speckle, Optical Imaging, Antimicrobial Susceptibility Testing (AST)

I. INTRODUCTION

One of the most significant advances in 20th century medicine was undoubtedly the discovery of antimicrobials, which were responsible for one of the greatest revolutions in terms of medical treatments [1-4]. However, as life have a great capacity to adapt and respond to changes in the environment, the abusive use of antimicrobials has warned the effectiveness of some antimicrobial agents. Faced with new challenges, bacteria rapidly developed antimicrobial resistance (AMR) by environmental adaptation, through Darwinian mechanisms of selection of the most fitted. Bacteria acquire AMR by gene mutation or incorporation of AMR genes transferred from other bacteria. It is thus possible to find bacterial strains that are able to resistant to various classes of antimicrobial agents. Such multidrug resistance (MDR) explains the current very worrisome situation worldwide of untreatable infections, which represent today one of the main public health problems [1, 2, 5-7]. As an example it is possible to mention the number of cases of high mortality associated to methicillin-resistant *Staphylococcus aureus* (MRSA) or decreased susceptibility to vancomycin (VISA) infections, as well as those derived from vancomycin-resistant enterococci (VRE), MDR strains of pneumococci, Gram-negative bacteria producing extended-spectrum beta-lactamases (ESBL) and meningococci with decreased susceptibility to penicillin.

The rational use of antimicrobials and the timely detection of their susceptibility are crucial to curb this problem. Antimicrobial susceptibility testing (AST) is performed after microorganisms are identified in samples (biological samples can be, for example, blood, saliva, urine, feces, and soft tissues) that are placed in a bacterial culture medium with ideal conditions for them to grow. Then the bacterial colonies are exposed to different types of antimicrobials and their ability to block growth is analyzed and the minimum amount of antimicrobial that allows such growth inhibition action is determined.

AST are in-vitro tests that identify whether an antimicrobial is effective against a particular bacterial strain and are the gold-standard in clinical practice for directing antimicrobial therapy. Their performance is mainly hindered by the time it takes to deliver results, which has great consequences in the management of antimicrobial targeted administration, with possible severe implications in the patient outcome and AMR and MDR emergence. In some cases the time required is about a week before results are obtained.

This paper presents results from the performance of a new AST that uses a technique based on the reflection of coherent light (in this case laser is used) and that allows obtaining results faster than the classic tests that require pre-incubation time in bacterial culture medium. After this first introductory section, this work continues with the Methodology. In section III the results are presented and in section IV the discussion. Finally, a brief conclusion is presented.

II. MATERIAL AND METHODS

A. Laser Speckle Imaging and Instrumentation

Speckle is an optical interference phenomenon obtained by diffused reflections when a rough surface is illuminated by a coherent light source. These reflected

randomly distributed waves create interference patterns that can be used to characterize static or dynamic conditions by using video processing algorithms [8, 9].

An illustration of the origin of the physical phenomenon of interference is shown in Figure 1. The light reflected by the surface being analyzed (in this case a petri dish with culture medium and a bacterial strain) is captured by a video recording system so that later, through digital image processing techniques, it is possible to associate movement patterns with the type of light interference that the camera's digital sensor can detect.

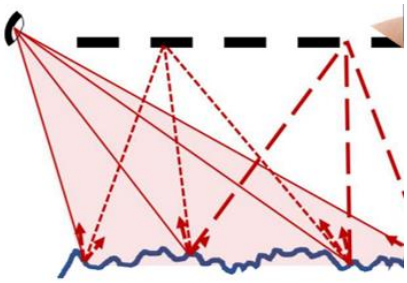

Figure 1: Illustration of the speckle phenomenon. The reflected wave fronts interact which others producing constructive or destructive interference pattern.

A sequence of 4 frames and an example of the region of interest (ROI) in one frame with the obtained pattern Speckle pattern is shown in Figure 2.

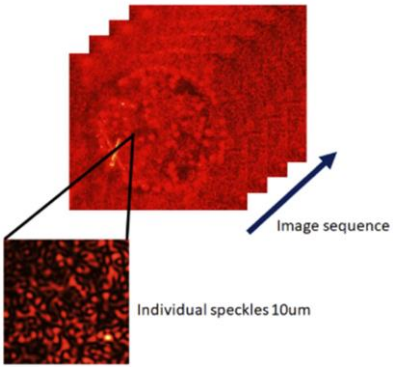

Figure 2: Sequence of frames and one ROI of the speckle pattern obtained from the raw data video.

The acquisition setup consists of a laser source (in this case a Ne-He laser with wavelength 633 nm and 5 mW power - Thorlabs), optical instrumentation (lens and diffuser) that allows the laser to cover a region of interest with sufficient area on the Petri dish, and a video system that allows for signal capture. The characteristics of the camera are described in Table 1 and the sketch of the setup is shown in Figure 4.

B. Experimental Procedure

For the experimental procedure, two bacterial strains (*Staphylococcus aureus* - S and *Pseudomonas aeruginosa* - P) and two antimicrobials (Imipenem - I and Ceftazidime - C) were employed. *Staphylococcus aureus* is a gram-positive bacteria that have a rounded shape and it is the only species in the genus *Staphylococcus* that have the enzyme coagulase. Other species that do not have this enzyme are called coagulase negative, and include some species that are also pathogenic, but less frequent, like *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. *Pseudomonas aeruginosa* is a gram negative bacilli with the capacity to produce several virulent extracellular substances and aggregate in biofilms, which further enhances its ability to acquire AMR and MDR mechanisms. Other species from this genus are also pathogenic, but to a much lesser extent.

TABLE I. CAMERA PARAMETERS

Parameters	acA1920-25gm
Maximum possible resolution	1920 x 1080
Color	Mono
Possible Synchronization Modes	Via Hardware trigger Via Software trigger Via Free run
Exposure time control	Via hardware trigger Programmable via the camera API
Camera Power Requirements	Via Ethernet connector - Power over Ethernet (PoE) 802.3af Via I/O connector - 12 VDC
I/O Lines	1 optocoupled input line 1 optocoupled output line

Regarding the antimicrobials used in this trial, imipenem is a beta-lactam antimicrobial from the carbapenem subgroup, and has a broad spectrum of activity against aerobic and anaerobic Gram-positive and Gram-negative bacteria, where tested strains are included. Ceftazidime is a beta-lactam antimicrobial from the 3rd generation cephalosporin group that shows increased activity against *Pseudomonas aeruginosa*, giving it a significant advantage over other cephalosporins to treat infections caused by MDR strains of this pathogen. Both antimicrobials are exclusively used in the hospital setting, only to treat MDR bacteria associated infections.

Each of the bacterial strains (S and P) were placed in culture medium in a petri dish divided into 3 circular sectors with different experimental conditions. In sector I only the bacterial strain was placed, in sector II the strain with the antimicrobial imipenem was placed, and in sector III the strain with the antimicrobial ceftazidime was placed. Then the petri dishes with the 3 experimental conditions were incubated for 30 minutes at 37 degrees Celsius. In the following, 10-second videos were recorded with a ROI within each circular sector of the plates at an acquisition frequency of 50 frames per second (fps). After recording, petri dishes were placed back in the incubator, and the recording procedure was repeated every 30 minutes for a total of 6 hours (data not shown).

III. RESULTS AND DISCUSSION

After being acquired, each of the videos went through a pre-processing phase, where the frames corresponding to each of the experimental conditions were selected and the ROI (in this case a 300 x 400 resolution rectangle) was defined. To quantify the activity in each of the ROIs, common statistical measures in laser speckle were used, in particular contrast and velocity descriptors. Image processing was performed using ImageJ software.

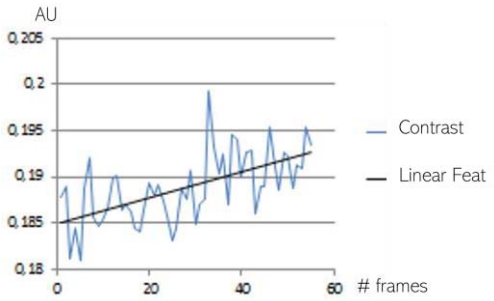

Figure 3: Contrast variation during 60 frames from one experimental condition.

One example of the contrast variation during 60 frames is shown in Figure 3. The linear fit was also plotted and the slope of the line was obtained as a measure for the intensity of the activity within the selected ROI for each period.

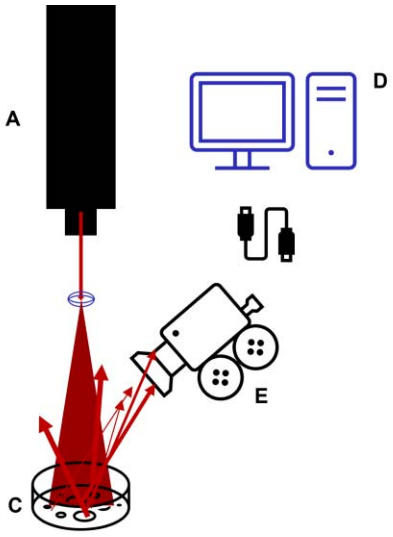

Figure 4: Illustration of the acquisition setup. A – Laser Source, B – Lens; C- Sample; D- Computer; E – Video system.

The results for each experimental conditions are shown in Table 2. The higher variation in the first 30 minutes is obtained for *Staphylococcus aureus* in the presence of imipenem, reporting an increase trend in the contrast which is compatible with reduced activity in the sample, thus showing susceptibility of *Staphylococcus aureus* to imipenem. This conclusion is in line with the expected, once imipenem has a broad spectrum of activity in particular against gram positives as is the case of *Staphylococcus aureus*.

In the remaining experimental conditions this trend is no longer registered, and is possible to observe a slight variation in the slope corresponding to a decrease in the contrast. This may be indicative of the antimicrobials consumption during the assays (since they are killing the bacteria), which is something to solve in our future experiments. We must include a step to introduce further doses of antimicrobials to attain constant concentrations.

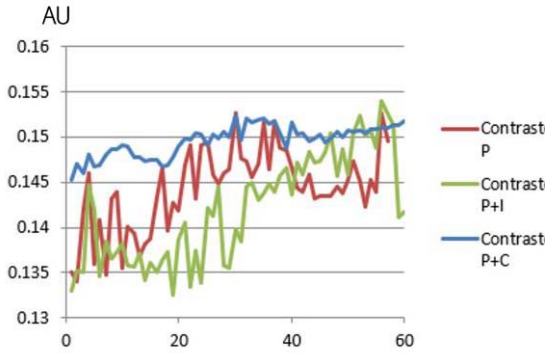

Figure 5: Contrast profile after 30 minutes of incubation for the three experimental conditions: *Pseudomonas aeruginosa* (P); *Pseudomonas aeruginosa* + imipenem (P+I); *Pseudomonas aeruginosa* + ceftazidime (P+C).

To better understand this effect of the decrease in contrast, Figure 5 shows the variation in the experimental conditions after 30 minutes, during 60 frames of acquisition. It is possible to see that the contrast values tend to be higher for *Pseudomonas aeruginosa* + ceftazidime, which indicates less activity detected, therefore, this result is compatible with the increased susceptibility of *Pseudomonas aeruginosa* to ceftazidime antimicrobial activity, described in literature.

TABLE II: CONTRAST AND VARIATION IN THE SLOPE FOR EACH EXPERIMENTAL CONDITION

A

Staphylococus Aureus + Imipenem		
Time in minutes	Contrast	Variation in the slope (***m***)
0	0.0001	0.1579
30	0.158	
Staphylococus Aureus + Ceftazidime		
Time in minutes	Contrast	Variation in the slope (***m***)
0	0.201	0.034
30	0.167	

B

Psudomonas A+ Imipenem		
Time in minutes	Contrast	Variation in the slope (***m***)
0	0.1681	0.057
30	0.1048	
Pseudomonas A+ Ceftazidime		
Time in minutes	Contrast	Variation in the slope (***m***)
0	0.1565	0.0381
30	0.1184	

IV. CONCLUSION

Antimicrobial resistance is a serious public health problem with worldwide expression. The misuse of antimicrobial therapy has contributed to the adaptation of bacteria and, therefore, to the development of resistance. The determination of antimicrobial susceptibility is a laboratory test that aids clinical practice, so that prescriptions can be made in a targeted manner, and as early as possible. However, in some cases AST may take up to a week to deliver results, which greatly impairs targeted antimicrobial prescription and thus favors AM and MDR emergence. In order to contribute to shorten AST time to deliver results, with this work we present an experimental study with a technique using coherent light to conduct AST, where results were found in half an hour.

Obtained results, although encouraging, need to be interpreted with some caution. In a short period of time it was possible to identify the reduction of activity in antimicrobial growth in the association of *Staphylococcus aureus* with imipenem, but in the other tested combinations the results were less expressive. In the case of the association of *Pseudomonas aeruginosa* with ceftazidime, although there was variation in the detected activity, this variation was reduced, indicating that the effect of antimicrobial therapy did not stop the growth of colonies in culture. It is known that some strains of *Pseudomonas aeruginosa* are resistant to third generation cephalosporins (such as ceftazidime), which could explain obtained results, since we did not know the susceptibility pattern of the used strain.

Another limitation of the study is related to the acquisition conditions, since the recordings were made in a laboratory with ambient light, and since laser light was used there may have been interference with the recordings. In future work, these recordings must also be performed over longer periods of time and in a more suitable location in terms of light conditions.

Further studies, with other bacterial growth under antimicrobials challenge conditions are required, as well as with other bacterial species and different antimicrobials. Moreover, experiments with MDR bacteria are also essential. Adjustments in antimicrobials concentrations during the assays were already pointed out in the text.

Nevertheless, these preliminary results point to a new, faster way to perform AST with the practical consequences already identified.

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