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Original article

Nanomechanical sensor applied to blood culture pellets: a fast approach to determine the antibiotic susceptibility against agents of bloodstream infections

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ABSTRACT

Objectives: The management of bloodstream infection, a life-threatening disease, largely relies on early detection of infecting microorganisms and accurate determination of their antibiotic susceptibility to reduce both mortality and morbidity. Recently we developed a new technique based on atomic force microscopy capable of detecting movements of biologic samples at the nanoscale. Such sensor is able to monitor the response of bacteria to antibiotic's pressure, allowing a fast and versatile susceptibility test. Furthermore, rapid preparation of a bacterial pellet from a positive blood culture can improve downstream characterization of the recovered pathogen as a result of the increased bacterial concentration obtained.

Methods: Using artificially inoculated blood cultures, we combined these two innovative procedures and validated them in double-blind experiments to determine the susceptibility and resistance of *Escherichia coli* strains (ATCC 25933 as susceptible and a characterized clinical isolate as resistant strain) towards a selection of antibiotics commonly used in clinical settings.

Results: On the basis of the variance of the sensor movements, we were able to positively discriminate the resistant from the susceptible *E. coli* strains in 16 of 17 blindly investigated cases. Furthermore, we defined a variance change threshold of 60% that discriminates susceptible from resistant strains.

Conclusions: By combining the nanomotion sensor with the rapid preparation method of blood culture pellets, we obtained an innovative, rapid and relatively accurate method for antibiotic susceptibility test directly from positive blood culture bottles, without the need for bacterial subculture. **P. Stupar, Clin Microbiol Infect 2017;23:400**

Introduction

Rapid acquisition of a blood culture pellet, coupled with a subculture-independent technique allowing phenotypic determination of the antibiotic susceptibility, could significantly reduce the time to results, greatly benefitting patient care and reducing cost of treatment, especially empiric treatments with broad-range antibiotics. A reduction of this timeline to less than one working day would be very useful, reducing morbidity and mortality as well as the overall costs for healthcare systems [1]. Furthermore, it would allow achieving faster treatments tailored to the patient, which would be more effective and better tolerated, with a reduced negative impact on beneficial bacteria.

To achieve this, we have proposed a new technique to rapidly characterize bacterial resistance to antibiotics [2–4]. This technique

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is based on the use of nanomechanical sensors [5–7] that oscillate if the attached bacteria are metabolically active. Oscillation detection is obtained using an optical lever method, where a laser beam is reflected off the cantilever and directed towards a detector (Fig. 1). Even among other nanomechanical sensor systems, this technique stands out as one of the most sensitive and fast in the study of bacterial growth and antibiotic susceptibility [8–13]. Nanomotion–antimicrobial susceptibility testing (AST) has several advantages compared to conventional culture-based systems commonly used in microbiology. For instance, it does not rely on bacterial growth; hence it is not negatively impacted by the replication time of the bacteria, and this reduces the characterization of the microorganism's resistance to minutes, compared to hours or days needed by conventional AST systems [14,15]. Furthermore, nanomotion-AST requires a low number of bacteria (approximately 10^2 bacteria) to obtain a measurable signal [3]. Overall, the speed and sensitivity of this technique allows an unprecedented insight on the bacterial response to environmental, chemical or physical stimuli.

Recently we developed an ammonium chloride–based blood culture pellet preparation test, which allows obtaining a high bacterial amount of an isolated strain for rapid downstream characterization [16]. This work demonstrates the feasibility of the previously described combination of bacterial pellets prepared from positive blood cultures [17] and nanomotion for rapid AST on *E. coli,* the most frequently recovered bacterial pathogen [18]. The findings of this study suggest that these two innovative techniques might serve as a versatile system, greatly affecting the field of microbiology. Their combination would ensure a significant reduction of the time needed to characterize bacteria from positive blood cultures to complete analysis in less than 3 hours.

Materials and methods

Reagents and antibiotics

All chemicals, phosphate-buffered saline (pH 7.4), Luria broth, glutaraldehyde and paraformaldehyde and the antibiotics ampicillin, ciprofloxacin, ceftriaxone and cefoxitin, all of analytical grade, were supplied by Sigma-Aldrich (St. Louis, MO, USA).

Bacterial preparation and isolation from blood cultures

Bacterial strains, and resistant and susceptible strains of *E. coli* were either American Type Culture Collection (ATCC) (Manassas, VA, USA) strains or clinical isolates of the bacterial repository of the Institute of Microbiology of the Lausanne University Hospital (Lausanne, Switzerland). The susceptible *E. coli* strain used for this study was ATCC 25922 (minimum inhibitory concentration (MIC) values for ceftriaxone, ciprofloxacin and ampicillin are 0.06, 0.008 and 8 μg/mL respectively). As resistant *E. coli* strain, we used a clinical isolate characterized using the VITEK device and Etest (bioMérieux, Marcy l'Etoile, France) methods, showing MIC values for ceftriaxone, ciprofloxacin and ampicillin as ≥64, ≥4 and ≥256 μg/mL respectively. Working concentrations of the antibiotics were chosen according to the European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org) interpretation guidelines to allow discrimination between resistant and susceptible strains.

Bacteria were inoculated together with human blood in anaerobic culture bottles (BACTEC Lytic/10 Anaerobic/F Medium; Becton Dickinson (BD), San Diego, CA, USA), recovered from the transfusion centre of our hospital, in BD blood vials and processed by a BACTEC FX automated blood culture system (BD). This system automatically detects the growth of microorganisms by detecting pH change due to CO_2 production. When detected positive, blood vials were processed in order to rapidly obtain a bacterial pellet; the method is described in detail elsewhere [17]. Briefly, 5 mL of the medium from a positive blood culture was mixed with 40 mL sterile water; after centrifugation (1000 × *g* for 10 minutes), the supernatant was removed, and the remaining blood cells were lysed by adding 1 mL ammonium chloride (0.15 M NH_4Cl, 1 mM $KHCO_3$, pH 7.31) to the bacterial pellet, followed by a second centrifugation step (140 × *g* for 10 minutes) to discard the lysed red blood cells.

Overall, this preparation protocol lasts less than 1 hour and ensures an enriched bacterial pellet that may be characterized. This bacterial pellet was finally resuspended in buffer and tagged with a code in order to ensure a blind susceptibility test with the nanomotion sensor.

Nanomotion-AST

To perform the susceptibility tests, we used both a homemade small, portable prototype device and a commercial JPK Nanowizard III microscope (JPK Instruments, Berlin, Germany) equipped with an Axiovert inverted optical microscope (Carl Zeiss GmbH, Jena, Germany) (Supplementary Fig. S1). The sensors we used in these experiments were commercial atomic force microscope cantilevers (NP-O10; Bruker Daltonics, Billerica, MA, USA). Sensor deflections were measured using the conventional laser-based system

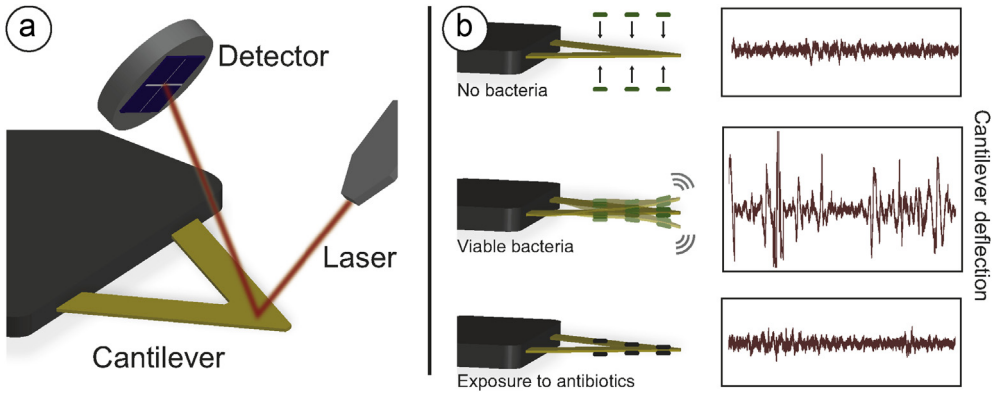

Fig. 1. Outline of experimental setup and description of experiments. (a) Schematics of nanomotion detector setup with cantilever sensor; laser beam is focused on surface of sensor, and reflection is used to monitor movements of cantilever. (b) Representation of typical nanomotion susceptibility test. When bacteria are not attached to sensor, fluctuations are driven only by thermal motion and are low. After attachment of live bacteria, fluctuations are linked to their metabolic activity and are high. Finally, after exposure to bactericidal drug, bacteria are nonviable and fluctuations return to low levels.