RAPID ANTIBIOTIC SUSCEPTIBILITY TEST: COMMERCIALIZATION OF LIFE SAVING MEMS DEVICES

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ABSTRACT

For the timely treatment of patients with infections in bloodstream and cerebrospinal fluid, a rapid antimicrobial susceptibility test (AST) is urgently needed. This paper describe a direct and rapid antimicrobial susceptibility testing (dRAST) system, which can determine the antimicrobial susceptibility of bacteria from a positive blood culture bottle (PBCB) in six hours that conventionally taking more than 30-50 hours. Design consideration, clinical verification, commercialization, and application of dRAST system to tuberculosis are reviewed.

INTRODUCTION

Bloodstream infection and tuberculosis have been regarded as a major global health problem, leading cause of morbidity and mortality among hospitalized patients. To prevent the emergence of antimicrobial resistance, the prescription of broad-spectrum antimicrobial drugs is managed by hospital antibiotic stewardship programs. Therefore, to improve the clinical prognosis in patients with bacteremia or tuberculosis, fast and accurate determination of the antimicrobial susceptibility of bacteria or mycobacteria is compulsory.

Although antibiotic susceptibility tests (ASTs) are common clinical procedures that help select appropriate antibiotic treatments, these tests take too long to complete. Because of the long lead times of ASTs, the initial antibiotic treatments are still mostly based on a physician's guess. The widespread overuse and misuse of antibiotics coupled with the decreasing number of new U.S. Food and Drug Administration (FDA)– approved antibiotics could result in global health challenges. Thus, it is important to reduce time for AST without compromising the accuracy of the tests.

EXPERIMENTAL

We have developed a rapid antibiotic susceptibility test (AST) system based on microfluidics, to reduce the total turnaround time of current antibiotic resistance detection process, as shown in Figure 1.[1] Specifically, we previously reported that tracking single cell growth in microfluidic channel determined drug susceptibility by calculation of bacteria-occupying area in the images. These tests determine antimicrobial susceptibility based on the simple observation of bacterial growth. However, the antimicrobial responses of bacteria are very heterogeneous and specific to different antibiotic conditions. It led to the hypothesis of more accurate characterization including morphological changes.

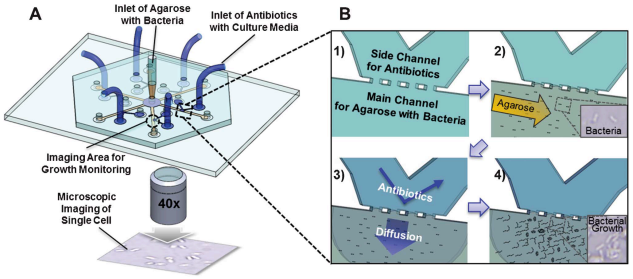

Figure 1: Schematic diagram of the antibiotic susceptibility testing process based on microfluidic agarose channel system. Different concentrations of antibiotics were supplied from the side-branched channels. Each interface between the agarose with bacteria and antibiotic solutions was monitored using a microscope to analyze bacterial cell growth.

Then, we proposed and demonstrated the rapid AST with imaging-based single-cell morphological analysis (SCMA) as a proof-of-concept of our previous hypothesis, shown in Figure 2. [2] SCMA can determine antimicrobial susceptibility by analyzing the morphological changes of single bacterial cells under various conditions. To adapt the SCMA to a high-throughput format, we developed a microfluidic chip that molds bacteria-mixed agarose to a thin, flat microscale slab. Thousands of morphological change patterns were acquired, performing time-lapse bright-field imaging of single cells using the microfluidic chip. From the results, we categorized the response of bacteria into several different morphological patterns. These patterns from our SCMA system were then compared with the clinical gold standard method.

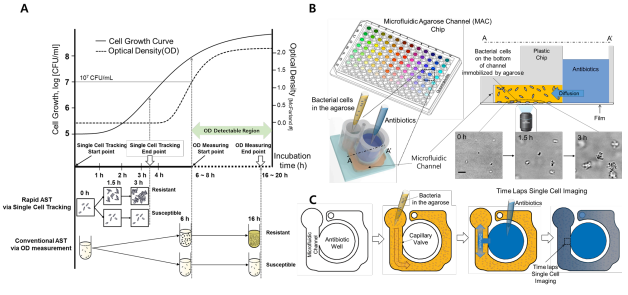

Figure 2: A) The comparison of AST based on single cell morphological analysis with the conventional method using optical density. B) The schematic structure of microfluidic agarose channel chip. This chip is integrated with the 96 well platform for high through analysis. (Scale bar represents 10um) C) Experimental procedure of AST utilizing MAC chip.

From both previous researches, we detected the antibiotic resistance of bacteria utilizing microscopic imaging rather than optical density (OD) measurement. For microscopic imaging of bacteria, we fixed both clinical sample derivatives in agarose matrix molded by a micro-patterned chamber. After fixation, we observed the bacterial at the single cell level, sequentially determining antibiotic resistance with help of an image processing system within 3 to 4 hours. Determination of bacterial susceptibility to antibiotics based on morphological assessment by human could be subject to human error. Therefore, we developed automated image-processing and classification program to automatically determine susceptibility of bacterial strains against antibiotics. Depending on six morphological change patterns, we successfully determined principal bacterial strains with high rates of categorical agreement in 3 to 4 hours. Based on these experimental results, we also developed direct AST system that can process positive blood culture samples without separation process, reducing about additional a day from conventional AST process.

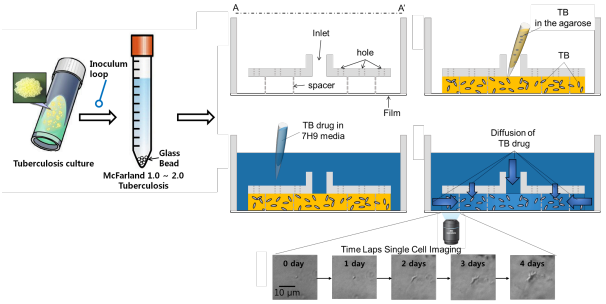

Figure 3: Experimental procedures of drug susceptibility test (DST) with mycobacterium tuberculosis (TB). After loading TB cells mixed with agarose matrix, anti-TB drug in liquid state is applied into micro-channel engraved microchip. Diffusion of drug and culture media and time-lapse imaging are conducted throughout days.

For application of our rapid AST system for other lethal infectious diseases, we developed drug susceptibility test (DST) system for mycobacterium tuberculosis (MTB), as shown in Figure 3. [3] Overall experimental method is similar to our rapid AST platform, however, due to the intrinsic growth feature of mycobacterium tuberculosis, we were able to assess drug susceptibility of MTB in 2 weeks with an accuracy to that of conventional method.

RESULTS AND DISCUSSION

We assessed the response of about 800 clinical pathogens representing various bacterial infections, including major antibiotic-resistant pathogens. In contrast to conventional OD based AST method, our system with image processing algorithms produced accurate results with highly reduced durations, fulfilling U.S. FDA requirements for accuracy requirements. Not only rapid AST results, we were able to produce concordant results in DST results, comparable to those obtained using conventional methods in 2 weeks.

CONCLUSION

Our rapid AST, DST test platform can determine bacterial susceptibility toward antimicrobial drugs faster than conventional AST and the gold standard method with same accuracy. This MEMS based technological platforms were validated with over 800 bacterial strains and over 400 mycobacterium tuberculosis strains. Based on these outcomes, we were confident that our rapid diagnostic system can be a help reducing global health problems related to infectious diseases. For broad clinical use, we are now developing fully automated system with sample preparation, image acquisition, and analysis.

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