RAPID ANTIBIOTIC SUSCEPTIBILITY TEST BASED ON THE MICROFLUIDIC AGAROSE CHANNEL WITH SINGLE CELL IMAGING PROCESS

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ABSTRACT

These days the most widely used methods for Antibiotics Susceptibility Test (AST) are the micro-dilution method for liquid condition and the disk diffusion method for solid condition. Using the micro-dilution method, automated AST systems have been developed. The system consume relatively large amount of bacteria and test time because it measures optical density (OD) for bacterial growth to determine the minimal inhibitory concentrations (MICs) of relevant antibiotics. We have invented a new microfluidic channel system for AST to reduce both amount of bacteria and test time dramatically. The agarose based microfulidic channel system fixes bacterial cell and tracks single cell growth to reduce AST assay time and to determine MICs of antibiotics. In this system, the conventional laboratory bacteria and four standard bacteria of Clinical and Laboratory Standard Institute (CLSI) were tested with several kinds of antibiotics to determine the MIC values. The obtained MIC values were confirmed using the conventional method (micro-dilution method) and also compared with the MIC data from CLSI. As a result, this system showed no different MIC values from conventional systems and innovatively reduced assay time and amounts of medium, antibiotics and bacterial cells for MIC determination

KEYWORDS

Antibiotic Susceptibility Test, Microfluidic Channel, Single Cell Imaging

INTRODUCTION

Owing to increasing antibiotic-resistant issues (e.g. MRSA and VRSA) [1], a rapid antibiotics susceptibility test (AST) is demanded. The most widely used methods for AST are microdilution or disk diffusion. Using the microdilution, automated AST instrument is developed as well [2]. These methods determine the minimal inhibitory concentration (MIC) of bacteria using optical density (OD) or macro scale change of bacterial colony. For these tests, large amount of bacteria and time (normally overnight) are needed.

We designed a microfluidic agarose channel (MAC) AST to reduce both amount of bacteria and test time (within few hours) by using the agarose based microfluidic channel system. The bacteria were injected to the microchannel with agarose. After applying media including different concentration of antibiotics in the microchannel, we checked the bacterial growth under microscope.

EXPERIMENT

The design of the microfluidic channel is in Figure 1. Using radial pattern of the channels, different concentrations of different antibiotics were applied for MIC test at the same time. The imaging process is below and Figure 1a. 1) The bacteria in preheated agarose were injected to the center of the channel and the bacterium spread out up to the branch point of the each channel in which the bacterium was fixed. 2) The several kinds of antibiotics were applied to the each channel with different concentrations in the cation adjusted Miller Hinton broth (CAMHB). The antibiotic solutions were diffused into the agarose so the bacterium was exposed to antibiotics. 3) The channels were incubated in the heat chamber. The bacterium did not grow over the MIC concentrations whereas it grew well under the MIC concentrations. 4) By measuring the growth of the bacterium under microscope and performing image processing (Fig. 2) for calculating the growth of the bacteria, we could determine the MIC of this bacterium.

RESULTS AND DISCUSSION

For example, MIC determination of amikacin against *P. aeruginosa* ATCC 27853 was performed in Figure 3. In the CLSI report, the MIC values are in the range of 1 to 4 μg/ml (Amikacin, *P. aeruginosa* ATCC 27853) Therefore, the concentrations of amikacin were determined in ranges of 0 to 8 μg/ml by two fold. After three hours of incubation, there were noticeable differences among the images. After image processing, we plotted the derived bacterial occupation data of bacterial growth on the graph according to incubation time. From the data, we determined the MIC values of amikacin against the *P. aeruginosa* ATCC 27853 (4 μg/ml). The 4 μg/ml of MIC values are in the MIC ranges of CLSI data[3]. In this system, the three standard bacteria of Clinical and Laboratory Standard Institute (CLSI) were tested with three kinds of antibiotics to be verified with the conventional AST system in table 1.

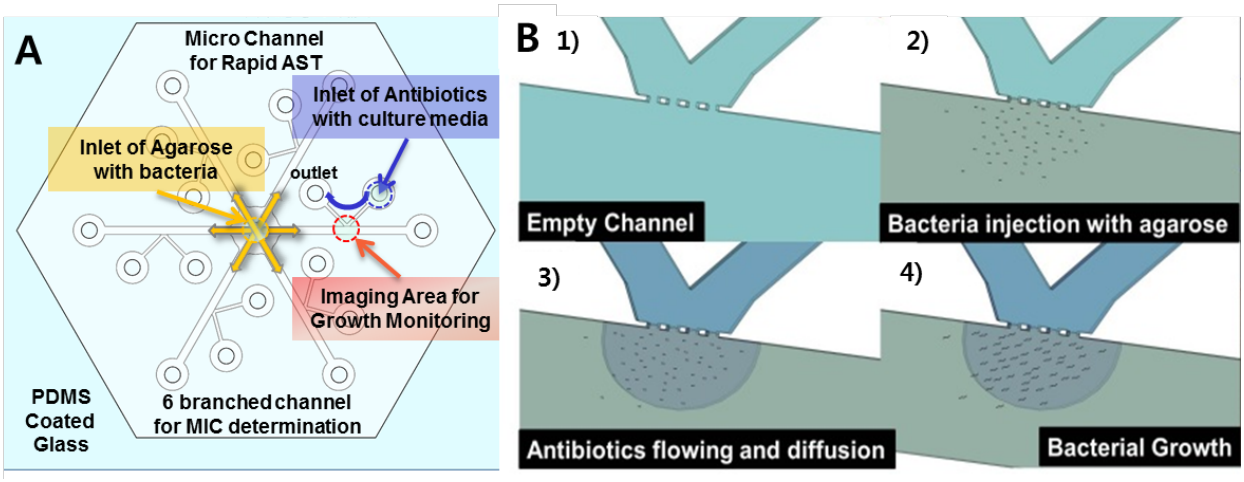

Figure 1. a) The schematic diagram for the microfluidic agarose channel (MAC) system. b-2) The agarose-bacterial mixture solution is injected at the center of the chip and applied into the six main channels synchronously. b-3) The different concentrations of antibiotic in the culture media are supplied from the side branched channels. b-4) Each interface between agarose with bacteria and antibiotics is monitored by microscopy for checking the growth. The process of the AST process of the MAC system. The bacterium is mixed with agarose and then injected into the main channels. The sharp interface is generated and liquid medium including different concentrations of antibiotic is applied from six side-branched channels into the main channel. The bacterial cell growth is tracked under microscope using time-lapse method.

Figure 2. Image processing. RGB images (a) were transformed to grey format images (b). The background was eliminated (c) and optimized (d). The processed images were changed to binary format images after enhancing imaging contrast (e).

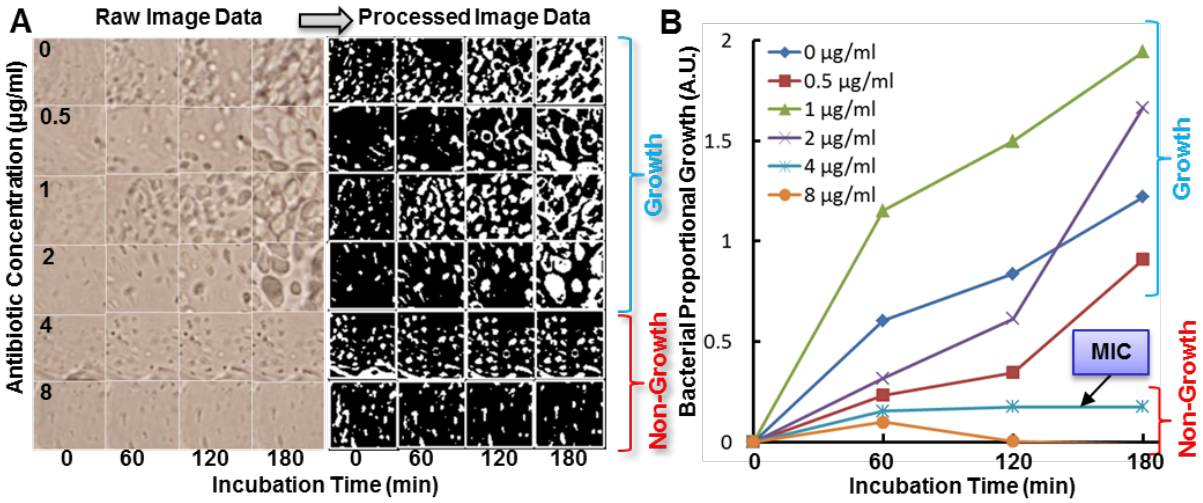

Figure 3. The bacterial growth and non-growth determination through image processing. Minimum Inhibitory Concentration (MIC) determination of amikacin against P. aeruginosa ATCC 27853. (A) The raw image data with incubation time and antibiotic concentration (left) and processed image data (right), respectively. (B) The graph of the bacterial proportional growth compared to the initial state with incubation time. MIC was determined to 4 μg/ml of amikacin against P. aeruginosa ATCC 27853.

Table 1. The comparison of the MIC from the CLSI and the MAC data. All MIC data of the MAC system were in the MIC ranges of CLSI results. (Unit : μg/ml)

Standard Strains / Antibiotics	*E.coli* ATCC 25922		*P.aeruginosa* ATCC 27853		*S. aureus* ATCC 29213	
	CLSI	MAC	CLSI	MAC	CLSI	MAC
Amikacin	0.5-4	4	1-4	4	1-4	4
Tetracycline	0.5-2	1	8-32	8	0.12-1	0.5
Gentamycin	0.25-1	1	0.5-2	2	0.12-1	1

CONCLUSIONS

In this research, we designed a microfluidic channel system called MAC which innovatively can save AST assay time. In the MAC system, the microchannel system is integrated with solidified agarose. The bacteria cells are fixed in the thin agarose matrix and different concentrations of antibiotics are supplied into the bacterial cells through diffusion method. The growth of bacterial single cell is tracked under microscope according to incubation time and the growth images are processed through our own image processing program to determine MIC values. The whole AST process requires 3~4 hours and the accuracy is comparable to the conventional AST results of CLSI.

In the clinical area, the MAC system can provide the rapid and accurate AST data for proper medication in clinical area. It would avoid the over or miss-use of the antibiotics and save many patients from death of sepsis.

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