Improved detection algorithms for label-free time-lapse cytometry of bacterial microcolonies for rapid antibiotic susceptibility testing

P. Yuen[1], C. Malmberg[1,2], P. Lagerbäck[1], O. Cars[1], T. Tängden[1]

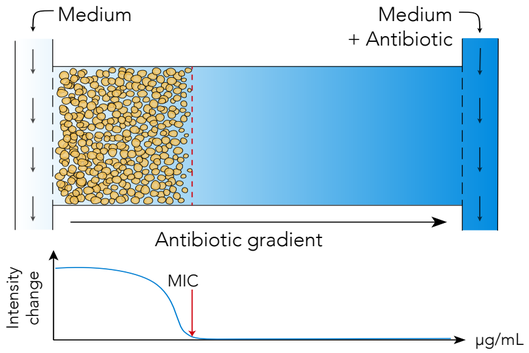
[1] Dept. of Medical Sciences, Uppsala University, Sweden
[2]Gradientech AB, Uppsala, Sweden

Background

- A novel microfluidic device CellDirector 3D (Gradientech AB) has previously been shown to determine the minimal inhibitory concentration (MIC) within 6 hours [1].
- The purpose of this study was to investigate the sensitivity and time to readout. Using improved detection algorithms and a new automated inverted brightfield microscope, the results were compared to intensity analysis and dark field microscope.

Experimental setup

A mixture of agarose and bacteria is injected into a chamber in the microfluidic device. Two channels, one filled with medium and antibiotic and the other one medium only, are flowing on opposite sides of the chamber. Due to diffusion, a linear antibiotic gradient is established in the device. The device is placed in a microscope with a 2x dark field condenser or in the oCelloScope. Bacterial growth is monitored using time-lapse photography and quantified by computation of the average intensity change along the antibiotic gradient axis or tracking of micro-colonies.



Correspondence to: pikkei.yuen@medsci.uu.se

Material and Methods

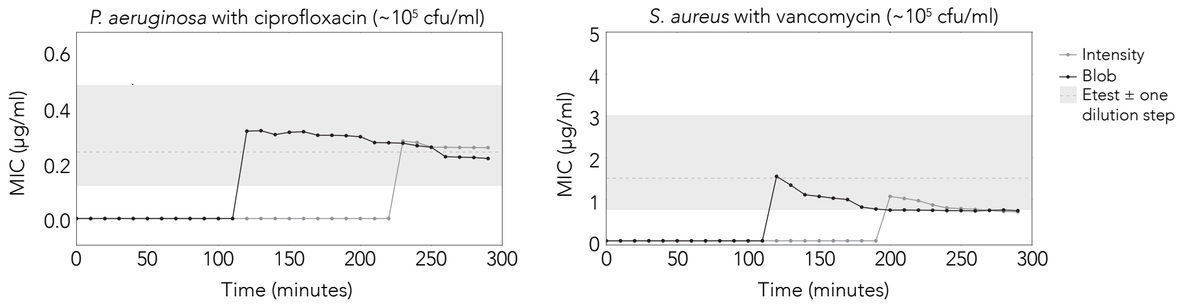
- Bacterial strains *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 29213) were tested using bacterial incolula of 10^3-10^6 cfu/ml.
- *P. aeruginosa* and *S. aureus* were exposed to ciprofloxacin (0-0.5 µg/ml) and vancomycin (0-4 µg/ml), respectively, for MIC determination.
- Time-lapse images of bacterial growth were captured automatically every 10 minutes during 5 hours using Nikon Optiphot-2 (Nikon, Japan) in 2x darkfield mode, or oCelloScope (Philips BioCell, Denmark) automated inverted brightfield microscope.
- The MIC was determined using either intensity analysis along the gradient axis or using blob analysis to track growing micro colonies.
- The results obtained using the microfluidic device were compared to the MICs determined using the Etest method (bioMérieux, France). Values within one dilution step from the Etest were considered as an acceptable variation.

- Inoculum size for a reliable detection of MIC using 2x dark field microscope (read at 300 min)

Strain / Antibiotic	Analysis	MIC (µg/mL) from CellDirector 3D (mean ± S.D., *n=3*) Inoculum size (cfu/ml) ~10^3	~10^4	~10^5	~10^6	MIC from Etest (µg/mL)
P. aeruginosa / ciprofloxacin	Intensity	ND	0.32 ± 0.005	0.35 ± 0.042	0.26 ± 0.014	0.25
	Blob	ND	0.24 ± 0.028	0.32 ± 0.057	ND	
S. aureus / vancomycin	Intensity	ND	0.90 ± 0.75	1.4 ± 0.47	2.0 ± 0.20	1.5
	Blob	3.7 ± 0.007	1.7 ± 1.7	1.1 ± 0.35	1.8 ± 0.64	

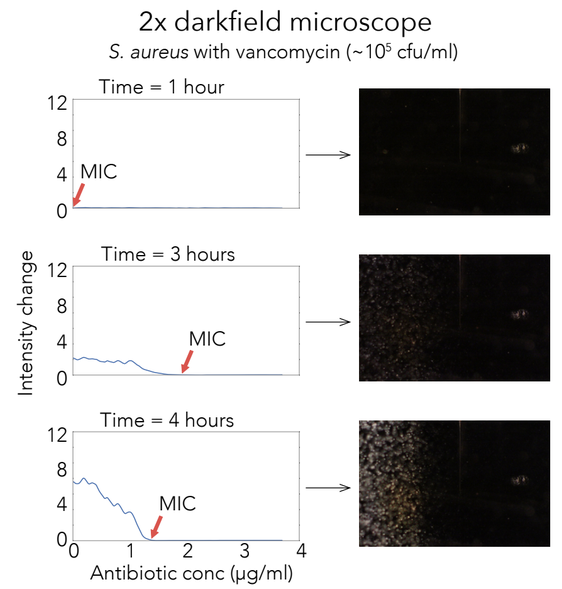
ND = Not determined

- Time to detection (example data from the oCelloScope)



Results and Conclusions

- The optimal inoculum size for a reliable MIC was 10^5 cfu/ml when analysing data using both intensity analysis and blob analysis.
- For *P. aeruginosa* and ciprofloxacin, the time to reliable MIC readout was improved for data collected in the oCelloScope and analysed using blob analysis; a mean of ~120 minutes vs a mean of ~200 minutes when using intensity analysis.
- For *S. aureus* and vancomycin, the time to MIC determination (~220 min) was limited by the establishment of the antibiotic gradient in the microfluidic device rather than bacterial growth and resolution.
- The results of MIC determination using both intensity analysis and blob analysis were consistent with results from Etest.



References:
[1] Hou Z, An Y, Hjort K, Hjort K, Sandegren L, Wu Z. Time lapse investigation of antibiotic susceptibility using a microfluidic linear gradient 3D culture device. Lab Chip. 2014;14: 3409. doi:10.1039/C4LC00451E